

1 **Evaluation of total and bioavailable heavy metals and other soil-**
2 **related variables in a rice paddy after the application of**
3 **defecation lime**

4 **Marcello Ermido Chiodini¹, Fulvia Tambone¹, Marco Carozzi², Mattia Sanna³, Silvia**
5 **Salati¹, Fabrizio Adani¹, Marco Acutis¹, Alessia Perego^{1*}**

6 ¹ Department of Agricultural and Environmental Sciences - Production, Landscape, Agroenergy,
7 Via Celoria 2, 20133, Milano, Italy

8 ² Agroscope Research Station, Climate and Air Pollution Group, Zurich, Switzerland

9 ³ Master Program in Global Health and Development, Taipei Medical University - Taipei City,
10 Taiwan

11 *Correspondence: alessia.perego@unimi.it tel.: +39 0250316611

12 **Summary:** A two-year experiment was carried out in a paddy field to investigate the effects
13 of the use of defecation lime derived from treated sewage sludge on soil total and soil
14 phytoavailable heavy metals concentration. Heavy metals concentration was determined also
15 in raw rice. Four treatments were arranged in a completely randomized block design: not
16 fertilised (T0), organic fertilisation + chemical fertilisers (T1), defecation lime + chemical
17 fertilisers (T2), defecation lime at pre-sowing (T3). For T3, the pH value increased
18 significantly at the end of the second year, increasing from 5.8 to 6.11. T3 resulted in the
19 highest soil organic carbon content (9.4 g kg⁻¹), suggesting the potential of defecation lime
20 both as soil corrective material and soil amendment. The application of defecation lime in the
21 paddy field did not result in an increased phytoavailable amount of heavy metals in soil.

22 **Keywords:** Defecation lime; Alkaline biosolid; Paddy soil; Rice; Heavy metals

23 **1. Introduction**

24 Sewage sludges are the sub-products derived from civil and industrial wastewater
25 treatment processes. According to various studies (GARCÍA *et al.*, 2000; MARTINEZ *et al.*,
26 2003; GARCÍA-GIL *et al.*, 2004; FERNÁNDEZ *et al.*, 2009), sewage sludge is defined as an
27 organic carbon-rich material produced during wastewater treatment and represents a source of
28 organic matter, nitrogen, phosphorus, and other nutrients. In the last decade, the production of
29 sewage sludges increased consistently in Europe because of the implementation of the
30 Council Directives 91/271/EEC and 98/15/EEC concerning urban wastewater treatment
31 (EUROPEAN COMMISSION, 2010); the European production of sewage sludges in 2009 was
32 approximately 10.13×10^6 Mg of dry matter (EUROPEAN COMMISSION, 2009). After their
33 production, sewage sludges can be stored in landfill, burnt or disposed of on agricultural land.
34 The EU directive 86/278/EEC encourages the application of sewage sludges in agriculture,
35 regulating their use to prevent environmental issues with soil, plants, animals and human
36 health. Moreover, land application of sewage sludges has been suggested to be the most
37 economical management method (GHANAVATI *et al.*, 2012). FROST and KETCHUM (2000)
38 reported that due to the high content of nutrients and stable organic matter, sludges have been
39 used in the past decades on the worldwide agricultural lands to improve soil fertility.

40 Despite their valuable effects, agricultural utilisation of sewage sludges can produce
41 negative impacts on the environment, especially in soil and water. These effects are due to the
42 presence of chemical contaminants, such as heavy metals and organic xenobiotics (TEIJON *et*

43 *al.*, 2010), and due to biological contaminants such as enteric parasites, viruses and pathogen
44 bacteria (SIDHU and TOZE, 2009). In fact, sewage sludges may contain high levels of toxic
45 metals, such as Ni, Cd, Zn, Cr, Cu, Pb, Hg and As (SINGH and AGRAWAL, 2007), besides
46 other harmful compounds such as pharmaceuticals, detergents, pesticides, organic
47 contaminants, flame retardants and hormone disruptors (SINGH and AGRAWAL, 2008).

48 The numerous metal contaminants in sewage sludge are either non-essential in plant
49 metabolism, such as Cd, Cr, Hg, Ni, and Pb, or essential in very low amounts, such as Cu, Fe
50 and Zn. The solubility and the bioavailability of trace metals from sewage sludge is based on
51 soil pH, soil cation exchange capacity, soil organic material and soil water-holding capacity
52 (JAMALI *et al.*, 2008). By increasing the pH of the sewage sludge, a reduction in trace metals
53 bioavailability occurs in soil.

54 Before land application, sludge stabilisation is needed in order to reduce its water content,
55 the emission of unpleasant odours and the concentration of pathogenic microorganisms.
56 Processing sludge with lime has been proposed for advanced sewage sludge treatment in the
57 relevant EU working document on sludge usage (SPINOSA, 2004). In fact, lime is considered
58 as one of the most common amendment materials for sewage sludge stabilisation, playing a
59 significant role in reducing microbial content, heavy metals availability and, at the same time,
60 enhancing agricultural benefits and lowering environmental risks (WONG and SELVAM, 2006).

61 The production of defecation lime implies lime stabilization, which is obtained by the
62 hydrolysis (and eventual enzymatic attack) of biological materials by using lime and/or

63 sulphuric acid and subsequent calcium sulphate precipitation (ITALIAN LEGISLATIVE DECREE
64 75/2010). Defecation lime is defined as a “calcium and magnesium corrective product” and it
65 must contain at least 20% of calcium oxide (CaO) and 15% of sulphuric anhydride (SO₃),
66 both on a dry matter basis. The available literature about defecation lime effects is still scarce
67 (MASHARIPOVA, 2006; GONZÁLEZ-FERNÁNDEZ *et al.*, 2004; SCIUBBA *et al.*, 2013); only
68 SCIUBBA *et al.* (2013) studied defecation lime produced from sewage sludge and no
69 information is yet available about its agronomic use and effects on agricultural land. This
70 study focused on defecation lime which was produced with sludges (i.e., industrial and urban
71 wastewaters) treated with SO₃ and CaO. This production aims at valorizing a product that
72 would otherwise require high costs for disposal. With the aim of evaluating the effectiveness
73 of the agronomic use of defecation lime in increasing soil pH and in decreasing the
74 bioavailability of heavy metals to rice, we set up a two-year experiment on a paddy field in
75 northern Italy under the hypothesis that defecation lime can be valuable as fertilizer and for
76 soil pH control.

77 **2. Materials and Methods**

78 *2.1. Experimental field and design*

79 The experiment was performed over two years (2009-2010), in a paddy field of 1.27 ha
80 located in the municipality of Cava Manara (Italy) (Lat. 45.151° N, Long. 9.084° E, Alt. 80 m
81 a.s.l.) in the Po valley, which is prone to non-point source pollution derived by slurry and
82 sewage sludge application on agriculture fields (FUMAGALLI *et al.*, 2013; PEREGO *et al.*,

83 2016). In the district of the field under study, rice (*Oryza sativa* L.) is widely cultivated. At
84 the beginning of the trial, soil had a moderate acid reaction and a loamy sand texture (USDA,
85 1993). A completely randomized block design with two blocks (Figure 1) was adopted to test
86 four treatments: i) not fertilised (T0), ii) common farmer paddy field management, which
87 includes organic fertilisation + chemical fertilisers (T1), iii) defecation lime + chemical
88 fertilisers (T2) and, iv) defecation lime (T3). Defecation lime in T2 and T3 and organic
89 fertilisers in T1 (hoof and horn, 150 kg ha⁻¹) were applied before tillage operation, while
90 chemical fertilisation in T1 and T2 treatments was carried out at two different rice
91 development stages: tillering (BBCH= 21), with ENTEC (24-0-29; 150 kg ha⁻¹), and panicle
92 initiation (BBCH= 30), with a N-P-K fertiliser (20-20-20; 375 kg ha⁻¹). Fertilisation
93 treatments were designed to provide similar amounts of nitrogen. T3 treatment was also
94 performed to evaluate the corrective ability of defecation lime on the soil. Each plot was 1260
95 m² (70 m long x 18 m width) and *Oryza sativa* L. cv. Volano (*Japonica* variety) was grown.
96 The area of each plot was set to allow the application of defecation lime by using a common
97 farm machinery. In this trial, a Franzosi FVR 110 muck spreader was used to apply defecation
98 lime, while a centrifugal fertilizer spreader was used to apply the other fertilizers. The
99 amounts of defecation lime applied in T2 were 2.4 Mg and 3.30 Mg dm ha⁻¹ in the first and in
100 the second year, respectively, while in T3 6.98 Mg and 13.98 Mg dm ha⁻¹ in the first and in
101 the second year were applied, respectively. The doses of defecation lime differed between T2
102 and T3 as T2 was meant to both fully satisfy crop N requirement and to increase soil pH,

103 whereas T3 was applied with the sole aim of evaluating the effectiveness of the defecation
104 lime to increase soil pH.

105 The rate of heavy metals added with other fertilizers was considered to be negligible.
106 Hoof and horn can be considered as a soil conditioner product, which is commonly used in
107 rice cultivation. This is considered to be a slow-release nitrogen product. This is derived from
108 cooked ground dehydrated cattle hooves and horns from slaughterhouses. Usually, N
109 concentration is 12-14% on a dry matter basis. Cu and Zn are the prevalent metals in the
110 product, whereas the others have negligible concentrations. In our experiment, the hoof and
111 horn content of Cu was 15.8 and Zn was 33 mg kg⁻¹ (certified organic fertilizer), respectively.
112 The actual rate of the Cu and Zn added to the soil was low if it is calculated considering the
113 hoof and horn application rate (150 kg ha⁻¹). ENTEC (24-0-29) is a slow release nitrogen
114 fertilizer. It is usually used for rice topdressing fertilization, specifically at the tillering stage,
115 both for its content of nitrogen and potassium. The N-P-K fertilizer (20-20-20) is a fertilizer
116 largely used in rice fertilization at the panicle initiation stage. No heavy metals are contained
117 in either the ENTEC or the N-P-K fertilizer.

118 The agronomic operations of rice cropping, namely fertilisation, sowing, weed control,
119 top dressing fertilisation, fungicide treatment and harvest, were similar over the years. The
120 paddy field was flooded by irrigation once a week, starting from three rice unfolded leaves
121 (BBCH 13). The irrigation was stopped one month before harvesting.

122 *2.2. Characteristics of the defecation lime*

123 The defecation lime used in the field trial was produced in a plant located at Bascapè
124 (Italy) and owned by Alan s.r.l. (Pavia, Italy). The production process (Figure 2) is based on
125 the European Patent Application EP 2 135 854 A2. The process is applied to sludge deriving
126 from industrial and municipal wastewater plants, and results in the stabilisation and sanitation
127 of the biological material, which can be consequently disposed on agricultural lands. The
128 chemical characteristics of the defecation lime used in the experiments were determined as
129 follows: a representative sample of up to 0.5 g was digested in 10 ml of concentrated nitric
130 acid (HNO₃) and hydrogen peroxide (H₂O₂) by microwave heating with a suitable laboratory
131 microwave unit (EPA, 1996). The sample and the acid solution were placed in a fluorocarbon
132 (PFA) microwave vessel. The vessel was capped and heated in the microwave unit for forty
133 minutes and then cooled to room temperature. After cooling, the vessel content was diluted to
134 a volume of 50 ml, filtered and then analysed. Elements concentrations were determined by
135 inductively coupled plasma mass spectrometry technique (ICP-MS, Varian, Fort Collins,
136 USA). A certified standard reference material (GBW 07405, soil) from the National Centre
137 for Standard Materials (Beijing, China) was used in digestion and analysis. To ensure
138 accuracy and precision, reagent blanks were run with samples.

139 *2.3. Soil sampling and characterisation*

140 Soil samples were collected from 0 to 25 cm depth by hand auger. Samples were
141 collected before the experiment for soil characterisation (first sampling) and at the end of the
142 two growing seasons: first year (second sampling) and second year (third sampling). At the

143 first sampling, the soil was sampled by randomly collecting eight soil cores in each block with
144 the aim of analysing the initial soil conditions before treatments. On the second and third
145 samplings, after treatments, soil was sampled from each plot. In particular, 8 samples were
146 collected in each plot and then bulked together to limit the variability. The samples were air-
147 dried, sieved to 2 mm mesh size and stored for analyses.

148 All soil samples were analysed for pH in water, cation exchange capacity (CEC; $\text{cmol}^{(+)}$
149 $\text{kg}^{-1} \text{ dm}$), total nitrogen (N_{tot} ; $\text{mg kg}^{-1} \text{ dm}$), total organic carbon (TOC; $\text{g kg}^{-1} \text{ dm}$), soil total
150 heavy metals concentration (Cr, Cu, Ni, Pb, Zn, Cd, Hg, As; $\text{mg kg}^{-1} \text{ dm}$) and plant
151 bioavailable heavy metals concentration (Cu, Ni, Zn and Cd; $\text{mg kg}^{-1} \text{ dm}$).

152 Common soil chemical parameters were determined, according to standard soil science
153 procedures (FAITHFULL, 2002). Total heavy metals concentrations were quantified as reported
154 for defecation lime characterisation.

155 Bioavailability was evaluated for four out of the eight heavy metals determined in the
156 soil. Cadmium was chosen because it is prone to be accumulated in rice and its intake can
157 cause several human diseases; for these reasons the control of the accumulation of Cd in
158 paddy soils is an important food-safety issue (URAGUCHI and FUJIWARA, 2012). Among the
159 other heavy metals, the bioavailability of Cu, Zn, and Ni was also studied because they
160 showed different levels of correlation between total and bioavailable concentrations (MA and
161 RAO, 1997).

162 Bioavailable heavy metals concentrations were quantified by inductively coupled plasma
163 mass spectrometry technique (ICP-MS, Varian; Fort Collins, USA). Before the analysis, a
164 specific extraction method was applied on soil samples, using an EDTA solution (ISO/DIS
165 14870, ISO 14876, ISO 11047). In detail, 8 g of an air-dried sample were shaken for about 30
166 minutes, after being mixed with 20 ml of EDTA, which was previously prepared by using
167 MilliQ deionized water (18 M Ω , MilliQ system, Millipore). Samples were then centrifuged at
168 5000 rpm for 5 minutes and the supernatants were moved to a 100 ml flask. Soils were
169 washed with MilliQ deionized water, centrifuged and supernatants moved again to the same
170 flasks. This washing cycle was repeated twice, and the solutions were raised to volume into
171 the flasks. At the end, the solutions were filtered by using Whatman No. 42 filter papers, and
172 the filtrates were used for the analyses at the ICP-MS, after adequate dilution.

173 *2.4. Statistical analysis*

174 To perform statistical analysis, a One-way ANOVA test by the IBM SPSS 20 software
175 was used. When significant differences were found ($P(f) < 0.05$), the Q-Ryan-Einot-Gabriel-
176 Welsch (R-E-G-W) test was used to identify homogeneous subsets.

177 **3. Results**

178 *3.1. Chemical characteristics of defecation lime*

179 Chemical characteristics of defecation lime are reported in Table 1. The concentration of
180 calcium oxide (CaO), sulfuric anhydride (SO₃), and total nitrogen (N_{tot}) presented similar

181 values over the two years, while the concentrations of heavy metals were notably lower than
182 the Italian law limits for corrective products (D.lgs. 75/2010).

183 *3.2. Soil chemical characteristics*

184 Soil physical and chemical characteristics estimated at the beginning of the experiment,
185 before treatments, are displayed in Table 2. As the soil was considered as homogenous before
186 treatments, the results of the analysis are shown as the average of the soil samples collected in
187 the 8 plots (2 Blocks x 4 Treatments). Texture was loamy sand (USDA), pH was slightly acid
188 (5.8), Total Organic Carbon (TOC, 8.19 g kg⁻¹) and Cation Exchange Capacity (CEC, 7.79
189 cmol⁽⁺⁾ kg⁻¹) had low values (ESDAC). A low value (0.66 g kg⁻¹) was also observed for total
190 nitrogen (N_{tot}).

191 The results collected during the experiment for the investigated soil variables are shown
192 in Table 3. All the soil variables, except for pH, showed not significant variations at the end
193 of the experiment. The small differences among treatments within the year and within the
194 treatment between the years suggest that the application of defecation lime did not modify soil
195 conditions and led to similar effects as the chemical fertilizer-based treatment, (i.e., T1). The
196 analyses highlighted differences in pH among treatments in the second year. pH increased
197 from 5.63 in T0 to 6.11 in T3 (Figure 3). The post-hoc test enabled us to split the treatments
198 into homogeneous subsets (i.e., T3^a, T0^b, T1^b, T2^b). At the end of the first year, T2 and T3
199 did not result in higher pH than the other treatments, whereas the second, higher dose of
200 defecation lime applied during the second year in T3 was able to increase soil pH. Regarding

201 the standard values of the lime amount required for soil correction, in this trial approximately
202 4.5 Mg ha⁻¹ of CaO would have been necessary for increasing pH from 5.8 (initial value in the
203 paddy soil; Table 2) to 6.11 (T3; Table 3).

204 3.3. Soil heavy metals concentration

205 3.3.1. Total soil concentration of heavy metals

206 The statistical analysis did not show differences in the total concentration of heavy metals
207 in soil among treatments within each year (Table 3). This was likely due to the low quantity
208 of metals supplied by defecation lime (Table 4). However, some differences could be noticed
209 when comparing the results of the first year with those of the second one. Total Ni and Cr
210 contents for all the treatments during the second year were higher than those observed in the
211 first year. Furthermore, on average, the values of Ni over the years were lower than those
212 recorded before the experiment (Table 2), unlike Cr. In the two years, Ni concentration
213 increased by 14%, 37%, 39% and 26% in T0, T1, T2 and T3, respectively. During the same
214 period, the Cr concentration increased by 25% in T0, while increases of 34%, 33% and 27%
215 were observed in T1, T2 and T3, respectively. Differences were also found for Zn: for all the
216 treatments the values in the first year were higher compared to the second year. For Hg just a
217 small difference was observed in T0 comparing the first to the second-year values. Overall,
218 Zn results were similar to the one reported before the experiment (Table 2). For Pb and As,
219 the trend increased from the first to the second year, except for As in T2. On average, the
220 values for these heavy metals were similar to the values determined before the experiment

221 (Table 2). No remarkable differences were observed for the other heavy metals suggesting no
222 effects following the application of defecation lime.

223 3.3.2. Bioavailable concentration of heavy metals in soil

224 The bioavailable concentrations of Ni, Cd, Zn and Cu are reported in Table 3. As shown,
225 no statistical differences were found over the experimental period. These results, as reported
226 above, could be due to the small amount of metals added by defecation lime application
227 (Table 4). Although the treatment effect was not statistically significant, small variations in
228 heavy metals concentrations were observed between the years. Variations in Zn soil
229 concentration were found in each treatment.

230 The determination of these elements in rough rice was carried out to validate the
231 concentration of bioavailable heavy metals observed in the soil (Table 5). The uptake which
232 occurred during rice growth could not be explored, since soil samples were collected only at
233 the end of the rice growing season. As expected, no statistical differences were found among
234 treatments, except for Cd concentration, for which significant differences ($P < 0.05$) were
235 found at the end of the second year. In particular, the homogenous subsets T3^a, T2^{a,b}, T1^b and
236 T0^b indicate that the plots treated with defecation lime resulted in a low concentration of Cd
237 in rice grains. Furthermore, as reported in Table 5, the concentrations of heavy metals during
238 the first year were 27% to 85% higher than the concentration measured in the second year.

239 4. Discussion

240 In the present work, we hypothesized that the defecation lime application, even at low to
241 medium rate, could increase soil pH, determining, as a side effect, an effect on heavy metal
242 bioavailability in rice. Accordingly, the results clearly showed an increase in pH from the first
243 to the second year in T3, in which the highest amount of defecation lime was added. We
244 remark that even if the applied defecation lime rate was low in the second year (3.43 Mg ha⁻¹),
245 it resulted in a significant increase in pH. The correction efficiency was 24% higher than the
246 tabulated value of CaO correction. A side effect of the pH increase was the low Cd
247 concentration in rough rice collected in the T3 treatment at the end of the second year.
248 Another positive effect of the defecation lime application is that it has a relevant cation
249 adsorption capacity (BOLAN *et al.*, 2003a) and can lead to the precipitation of Cd as CdCO₃
250 (HOLM *et al.*, 2003); this causes a reduction in the availability of Cd to plants. Alongside this
251 aspect, the use of defecation allows the application of organic matter to soil, which is of great
252 importance especially in rice paddy fields where soil organic matter is usually low. The
253 organic matter of defecation lime was likely to affect Cd adsorption. In this study, the highest
254 value of TOC was observed in T3 at the end of the second year. CATTANI *et al.* (2008)
255 reported that the application of composted material with lime produced an effect on Cd
256 assimilation by rice, reducing its bioavailability, which was undetected in the case of
257 application of composted material alone and more similar to that of lime alone.

258 Moreover, in this study the limited concentration of heavy metals evaluated over the
259 years can be due to the peculiar conditions of the paddy field, where water is heavily used

260 and, as a consequence, some heavy metals could be transported and moved away. A decrease
261 in heavy metals concentration in the soil due to water run-off and leaching was observed by
262 WANG *et al.* (2003). In this trial the variations observed for Zn over each treatment were
263 probably due to its high solubility (KABATA-PENDIAS, 2011) and related to the amount of the
264 element transported into the paddy field from water movements. In our experiment, the paddy
265 field was flooded by irrigation once a week, starting from the phenological stages of three rice
266 unfolded leaves, before tillering, until one month before harvesting.

267 In this work, the redox (Eh) conditions of the soil were able to influence heavy metal
268 availability. As reported before, the irrigation of the experimental paddy field had a
269 management similar to the Water Saving Irrigation (WSI) technique, which involves multiple
270 wetting-drying cycles of the paddy field and high Eh conditions (MAO, 2002; BOUMAN,
271 2007). As reported by XU *et al.* (2013), wetting-drying cycle and the high Eh conditions in
272 WSI paddy fields lead to modifying the solubility of heavy metals in soil and consequently
273 influenced plant uptake. Under flooded conditions, usually corresponding to an Eh lower than
274 -200 mV, sulfate ions are reduced to sulfide ions that may form complexes with Cd and other
275 metals, immobilizing them as sulphide salts (e.g. CdS, GIMENO-GARCÌA *et al.*, 1996).

276 Although the water level of the field during the two years was not measured, it can be
277 assumed that during the first year Cd was less available because of the low rainfall during the
278 experimental period. In the first year, the amount of rainfall from rice emergence to harvest
279 was 76 mm, while the amount was 235 mm in the second year during the same period. From

280 the end of flowering to harvest rainfall was 35 mm and 78 mm in the first and in the second
281 year, respectively. As a consequence, it is likely that soil conditions in the second year were
282 more reductive, thus leading to unavailable or less available Cd forms. The short duration of
283 the experimental trial could be the reason for the small variation observed in the data. GASKIN
284 *et al.* (2003) found that the water-soluble concentrations of Cu, Cd, Mo, Ni, and Zn increased
285 significantly in medium-term sludge application (>6 years). Considering the Cd content
286 observed in the grain collected in the experiment, in some cases the concentration exceeded
287 the limit of 0.20 mg kg⁻¹ (EUROPEAN COMMISSION, 2006). However, since this maximum
288 level admitted refers to polished rice grains, while the measurements performed in this study
289 were carried out on rough rice, it is not possible to state that the observed values exceeded this
290 level.

291 **5. Conclusions**

292 The results obtained from the two-year experiment indicate that this trial provides
293 suitable to a preliminary assessment of the effects of defecation lime on the chemical
294 characteristics of paddy soil. The outcomes of this experiment suggest that defecation lime
295 has the potential to affect soil pH and, according to the collected data, to be more effective
296 than CaO in correcting soil pH.

297 The bioavailability of heavy metals showed no variations following the use of defecation
298 lime, possibly due to (i) the low amount of metals contained in the defecation lime applied,
299 (ii) the characteristic of the growing system (paddy field) where water could strongly

300 influence heavy metals mobility and bioavailability into the agroecosystem, and (iii) the short
301 term of the trial.

302 Based on the results of this study and to better understand the effect of this product,
303 further research should be conducted with the following objectives: i) compare different
304 liming products to test the corrective capacity of pH of the defecation lime, ii) monitor the
305 trend of heavy metals concentration over the entire growing season to keep under control their
306 availability and their amount in rice plant and grain, iii) determine the redox status of the soil,
307 and iv) quantify and characterize the soil dissolved organic matter before and after the
308 addition of defecation lime.

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402 **Tables**

403 **Table 1.** Chemical characteristics of the defecation lime applied in the present
404 study. Chemical characteristics are as percentage of dry matter; “Limit” represent
405 the maximum concentration of heavy metals allowed for corrective products by
406 law (D.lgs 75/2010); n.d. = not defined by law for these elements; tot = total
407 amount. \pm Mean standard error.

| Variable | 1 st year | 2 nd year | Limit (d.lgs 75/2010) |
|-------------------------------|----------------------|----------------------|-----------------------|
| Dry matter (%) | 27.5 | 35.0 | |
| CaO (%) | 26.5 | 24.7 | > 20 % |
| SO ₃ (%) | 18 | 20.1 | > 15 % |
| TOC (%) | 29.1 | 32.0 | |
| N tot (%) | 2.8 | 2.3 | |
| Ni_tot (mg kg ⁻¹) | 22 \pm 2 | 58.98 \pm 5.83 | 100 |
| Cd_tot (mg kg ⁻¹) | 0.5 \pm 0 | 0.46 \pm 0.05 | 1.5 |
| Zn_tot (mg kg ⁻¹) | 390 \pm 70 | 213.25 \pm 35.14 | 500 |
| Hg_tot (mg kg ⁻¹) | 0.72 \pm 0.13 | 0.07 \pm 0.02 | 1.5 |
| Cu_tot (mg kg ⁻¹) | 130 \pm 13 | 70.43 \pm 8.18 | 230 |
| Pb_tot (mg kg ⁻¹) | 29.7 \pm 2.4 | 57 \pm 7.0 | 140 |
| Cr_tot (mg kg ⁻¹) | 53.5 \pm 5.4 | 55.8 \pm 5.3 | n.d. |
| As_tot (mg kg ⁻¹) | 0.4 | 3.5 | n.d. |

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Table 2: Soil chemical characteristics at the beginning of the experiment before the fertilizers' application. tot = total; Heavy metals are reported as total concentration of soil dry matter; Italian law limits imposed by D.Lgs 99/92 and by 86/278/EEC are referred of sludge-treated soil because defecation lime are not subjected to any application restraint; n.d. = not defined by law. \pm Mean standard error.

| Parameter | Value | Limit (D.Lgs. 99/92) (86/278/EEC) |
|--|-----------------|---|
| pH _{H2O} | 5.8 \pm 0.03 | |
| Texture (USDA) | loamy sand | |
| Sand (%) | 79.9 | |
| Silt (%) | 16.3 | |
| Clay (%) | 3.8 | |
| TOC (g kg ⁻¹) | 8.19 \pm 1.5 | |
| CEC (c mol ⁽⁺⁾ kg ⁻¹) | 7.79 \pm 0.15 | |
| N _{tot} (g kg ⁻¹) | 0.66 \pm 0.03 | |
| Ni _{tot} (mg kg ⁻¹) | 23.9 \pm 1.7 | 75 |
| Cd _{tot} (mg kg ⁻¹) | 0.1 \pm 0.0 | 1.5 |
| Zn _{tot} (mg kg ⁻¹) | 33.9 \pm 0.8 | 300 |
| Cu _{tot} (mg kg ⁻¹) | 8.9 \pm 1.2 | 100 |
| Cr _{tot} (mg kg ⁻¹) | 20.2 \pm 0.5 | n.d. |
| Hg _{tot} (mg kg ⁻¹) | 0.07 \pm 0.02 | 1 |
| Pb _{tot} (mg kg ⁻¹) | 16.8 \pm 0.0 | 100 |
| As _{tot} (mg kg ⁻¹) | 2.65 \pm 0.00 | n.d. |

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446 **Table 3.** Soil chemical characteristics observed at the end of each year of the experiment. All values are reported on dry matter basis. “tot” is the total heavy
 447 metals concentration; “bio” is the bioavailable heavy metals concentration. “bio/tot” is the ratio of bioavailable to total heavy metals. T0=unfertilised,
 448 T1=common farmer paddy field management including pre-sowing organic fertilisation (hoof and horn) and chemical fertiliser, T2=defecation lime +
 449 chemical fertiliser, T3=defecation lime at pre-sowing. *world-soil average is derived from Kabata-Pendias, 2011. ± Mean standard error.

| | Treatment | | | | | | | | World-soil average* |
|-------------------------------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|---------------------|
| | T0 | | T1 | | T2 | | T3 | | |
| | Year | | | | | | | | |
| | 1 st year | 2 nd year | 1 st year | 2 nd year | 1 st year | 2 nd year | 1 st year | 2 nd year | |
| pH (H ₂ O) | 5.95 ± 0.07 | 5.63 ± 0.19 | 5.90 ± 0.10 | 5.65 ± 0.09 | 5.70 ± 0.10 | 5.78 ± 0.03 | 5.80 ± 0.10 | 6.11 ± 0.06 | |
| TOC (g kg ⁻¹) | 7.90 ± 0.80 | 6.53 ± 1.14 | 8.78 ± 0.92 | 8.47 ± 1.43 | 7.60 ± 0.40 | 7.34 ± 1.69 | 8.53 ± 0.05 | 9.41 ± 0.59 | |
| CEC (cmol kg ⁻¹) | 7.61 ± 0.98 | 5.96 ± 0.41 | 7.51 ± 1.42 | 6.58 ± 1.76 | 7.90 ± 2.00 | 5.99 ± 0.36 | 8.16 ± 1.36 | 8.88 ± 0.51 | |
| N tot (g kg ⁻¹) | 0.61 ± 0.16 | 0.75 ± 0.12 | 0.61 ± 0.13 | 0.78 ± 0.01 | 0.69 ± 0.16 | 0.78 ± 0.05 | 0.74 ± 0.03 | 0.90 ± 0.01 | |
| Ni_tot (mg kg ⁻¹) | 14.6 ± 0.28 | 16.94 ± 1.25 | 12.08 ± 2.43 | 19.19 ± 1.95 | 11.8 ± 0.4 | 19.20 ± 1.80 | 13.85 ± 1.48 | 18.81 ± 2.56 | 29.0 |
| Cd_tot (mg kg ⁻¹) | 0.15 ± 0.02 | 0.15 ± 0.02 | 0.15 ± 0.03 | 0.12 ± 0.02 | 0.16 ± 0.06 | 0.14 ± 0.03 | 0.19 ± 0.03 | 0.15 ± 0.04 | 0.4 |
| Zn_tot (mg kg ⁻¹) | 32.3 ± 2.83 | 30.11 ± 2.54 | 31.9 ± 1.2 | 27.41 ± 1.49 | 31.75 ± 0.07 | 32.2 ± 3.5 | 33.8 ± 0.99 | 32.90 ± 3.30 | 70.0 |
| Cu_tot (mg kg ⁻¹) | 7.25 ± 0.64 | 7.02 ± 0.43 | 7.25 ± 0.78 | 6.8 ± 0.70 | 6.95 ± 0.64 | 6.53 ± 0.55 | 6.97 ± 0.09 | 6.97 ± 0.47 | 39.0 |
| Cr_tot (mg kg ⁻¹) | 17.95 ± 1.06 | 23.82 ± 1.48 | 17.75 ± 0.49 | 26.8 ± 2.8 | 17.9 ± 1.13 | 26.62 ± 3.17 | 19.56 ± 0.64 | 26.66 ± 3.31 | 60.0 |
| Hg_tot (mg kg ⁻¹) | 0.07 ± 0.01 | 0.13 ± 0.07 | 0.06 ± 0.0 | 0.08 ± 0.05 | 0.07 ± 0.0 | 0.08 ± 0.02 | 0.07 ± 0.01 | 0.06 ± 0.01 | 0.1 |
| Pb_tot (mg kg ⁻¹) | 15.35 ± 2.47 | 16.93 ± 2.61 | 16.85 ± 0.49 | 17.27 ± 1.23 | 16.2 ± 0.8 | 17.74 ± 0.21 | 17.3 ± 0.28 | 19.38 ± 1.82 | 27.0 |
| As_tot (mg kg ⁻¹) | 2.44 ± 0.08 | 2.78 ± 0.26 | 2.49 ± 0.44 | 2.93 ± 0.23 | 2.8 ± 0.3 | 2.6 ± 0.1 | 2.48 ± 0.11 | 2.94 ± 0.27 | 6.8 |
| Ni_bio (mg kg ⁻¹) | 0.12 ± 0.02 | 0.13 ± 0.02 | 0.12 ± 0.00 | 0.13 ± 0.01 | 0.13 ± 0.00 | 0.13 ± 0.00 | 0.13 ± 0.01 | 0.13 ± 0.01 | |
| Cd_bio (mg kg ⁻¹) | 0.02 ± 0.00 | 0.02 ± 0.00 | 0.03 ± 0.00 | 0.02 ± 0.00 | 0.03 ± 0.00 | 0.02 ± 0.00 | 0.03 ± 0.00 | 0.02 ± 0.00 | |
| Zn_bio (mg kg ⁻¹) | 1.11 ± 0.14 | 1.20 ± 0.10 | 1.32 ± 0.31 | 1.16 ± 0.05 | 1.15 ± 0.04 | 1.14 ± 0.05 | 1.24 ± 0.02 | 1.36 ± 0.03 | |
| Cu_bio (mg kg ⁻¹) | 0.58 ± 0.07 | 0.75 ± 0.23 | 0.47 ± 0.05 | 0.78 ± 0.06 | 0.49 ± 0.14 | 0.69 ± 0.01 | 0.48 ± 0.00 | 0.67 ± 0.06 | |
| Ni_bio/tot (%) | 0.82 | 0.77 | 0.99 | 0.68 | 1.1 | 0.68 | 0.94 | 0.69 | |
| Cd_bio/tot (%) | 13.33 | 13.33 | 20.00 | 16.67 | 18.75 | 14.29 | 15.79 | 13.33 | |
| Zn_bio/tot (%) | 3.44 | 3.99 | 4.14 | 4.23 | 3.62 | 3.54 | 3.67 | 4.13 | |
| Cu_bio/tot (%) | 8.00 | 10.68 | 6.48 | 11.47 | 7.05 | 10.57 | 6.89 | 9.61 | |

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Table 4. Amount of heavy metals added with defecation lime and increase of soil metals' concentration over the experiment. T2=defecation lime + chemical fertiliser, T3=defecation lime at pre-sowing.

| | | Metals | | | | | | | | | | | | | | | |
|---|---------------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|
| | | Ni | | Cd | | Zn | | Cu | | Cr | | Hg | | Pb | | As | |
| | | Year | | | | | | | | | | | | | | | |
| | Treatmen t | 1 st year | 2 nd year | 1 st year | 2 nd year | 1 st year | 2 nd year | 1 st year | 2 nd year | 1 st year | 2 nd year | 1 st year | 2 nd year | 1 st year | 2 nd year | 1 st year | 2 nd year |
| Heavy metals added with defecation lime [mg soil kg ⁻¹] ^a | 2 | 0.02 | 0.09 | 0 | 0 | 0.27 | 0.34 | 0.09 | 0.11 | 0.04 | 0.09 | 0 | 0 | 0.02 | 0.09 | 0 | 0.01 |
| | 3 | 0.04 | 0.39 | 0 | 0 | 0.78 | 1.41 | 0.26 | 0.47 | 0.11 | 0.37 | 0 | 0 | 0.06 | 0.38 | 0 | 0.02 |
| Increase of soil metals' concentration over the two-year experimental period [%] ^b | 2 | 0.06 | 0.39 | 0.34 | 0.73 | 0.79 | 1.00 | 1.00 | 1.26 | 0.18 | 0.44 | 0.71 | 0.15 | 0.12 | 0.54 | 0.01 | 0.21 |
| | 3 | 0.18 | 1.63 | 1.00 | 3.04 | 2.30 | 4.16 | 2.91 | 5.23 | 0.53 | 1.83 | 2.05 | 0.61 | 0.35 | 2.24 | 0.03 | 0.88 |

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^a Defecation lime rate (t ha⁻¹ d.m.) was: 2.40 (T2), 6.98 (T3) in 2009; 3.33 (T2), 13.98 (T3) in 2010. ^b Initial soil concentration is given in Table 2. Soil weight was calculated by multiplying bulk density (1.4 Mg m⁻³) by surface area (10000 m²) by tillage depth (0.25 m in 2009 while 0.15 m in 2010).

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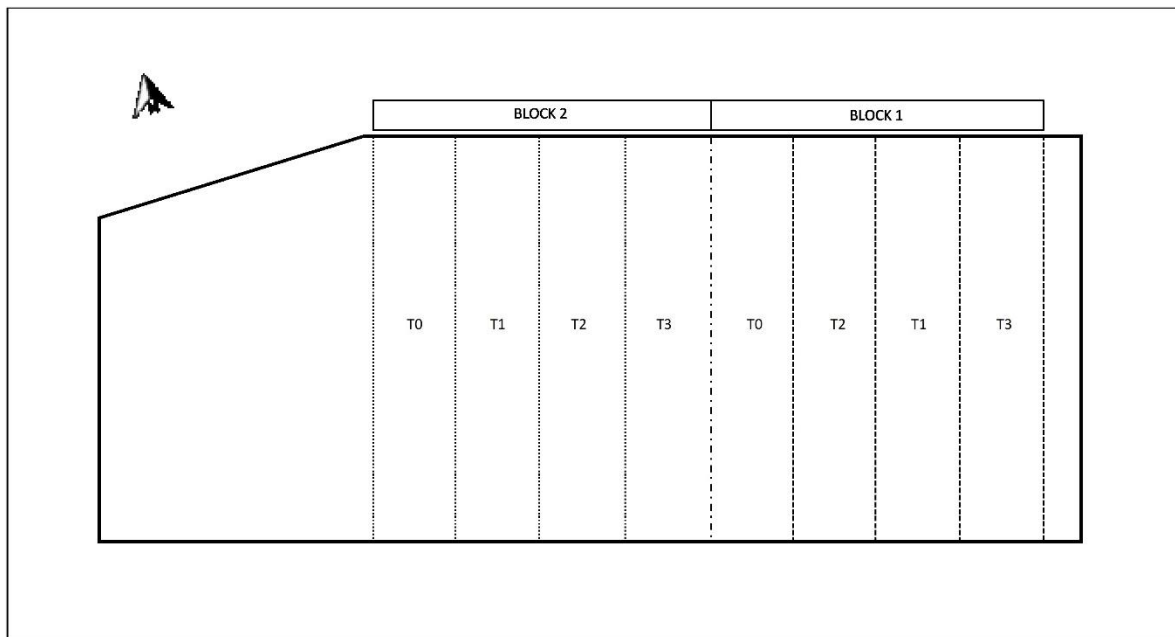
Table 5. Heavy metals total concentration in rough rice at the harvest. T0=unfertilised, T1=common farmer paddy field management including pre-sowing organic fertilisation (hoof and horn) and chemical fertiliser, T2=defecation lime + chemical fertiliser, T3=defecation lime at pre-sowing. Concentrations are reported on dry matter basis. Different letters indicate statistically significant differences of Cd between treatments in the second experimental year (Q-Ryan-Einot-Gabriel-Welsch post-hoc test).

| Heavy Metals [mg kg ⁻¹] | Treatment | | | | | | | |
|-------------------------------------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|
| | T0 | | T1 | | T2 | | T3 | |
| | 1 st year | 2 nd year | 1 st year | 2 nd year | 1 st year | 2 nd year | 1 st year | 2 nd year |
| Cd_tot | 0.35 ± 0.03 | 0.25 ± 0.01a | 0.29 ± 0.03 | 0.23 ± 0.02a | 0.30 ± 0.02 | 0.16 ± 0.03a,b | 0.29 ± 0.10 | 0.11 ± 0.02b |
| Ni_tot | 14,84 ± 3,61 | 13,33 ± 2,42 | 13,87 ± 1,33 | 15,49 ± 0,09 | 8,94 ± 1,34 | 8,43 ± 0,79 | 6,49 ± 0,92 | 6,54 ± 1,52 |
| Zn_tot | 22.33 ± 1.38 | 17.45 ± 0.25 | 23.60 ± 2.33 | 18.35 ± 1.10 | 24.61 ± 0.93 | 16.86 ± 0.24 | 23.82 ± 2.51 | 17.24 ± 0.18 |
| Cu_tot | 3.70 ± 0.30 | 3.14 ± 0.06 | 3.87 ± 0.66 | 3.05 ± 0.10 | 4.10 ± 0.02 | 2.79 ± 0.12 | 4.14 ± 0.11 | 3.11 ± 0.11 |
| Cr_tot | 31.75 ± 7.87 | 19.27 ± 3.68 | 28.07 ± 6.27 | 17.56 ± 0.93 | 29.26 ± 3.79 | 13.85 ± 1.88 | 33.52 ± 1.59 | 14.47 ± 2.55 |
| Hg_tot | 0.01 ± 0.01 | 0.00 ± 0.01 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.01 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| Pb_tot | 0.18 ± 0.08 | 0.08 ± 0.01 | 0.17 ± 0.07 | 0.07 ± 0.02 | 0.13 ± 0.00 | 0.06 ± 0.02 | 0.12 ± 0.00 | 0.07 ± 0.00 |
| As_tot | 0.93 ± 0.19 | 0.26 ± 0.02 | 0.83 ± 0.22 | 0.25 ± 0.03 | 0.78 ± 0.13 | 0.22 ± 0.02 | 0.71 ± 0.25 | 0.19 ± 0.03 |

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473 **Figures captions**

474 **Figure 1.** Schematic view of the experimental area. T0=unfertilised, T1=common farmer
475 paddy field management including pre-sowing organic fertilisation (hoof and horn) +
476 chemical fertiliser, T2=defecation lime + chemical fertiliser, T3=defecation lime at pre-
477 sowing.



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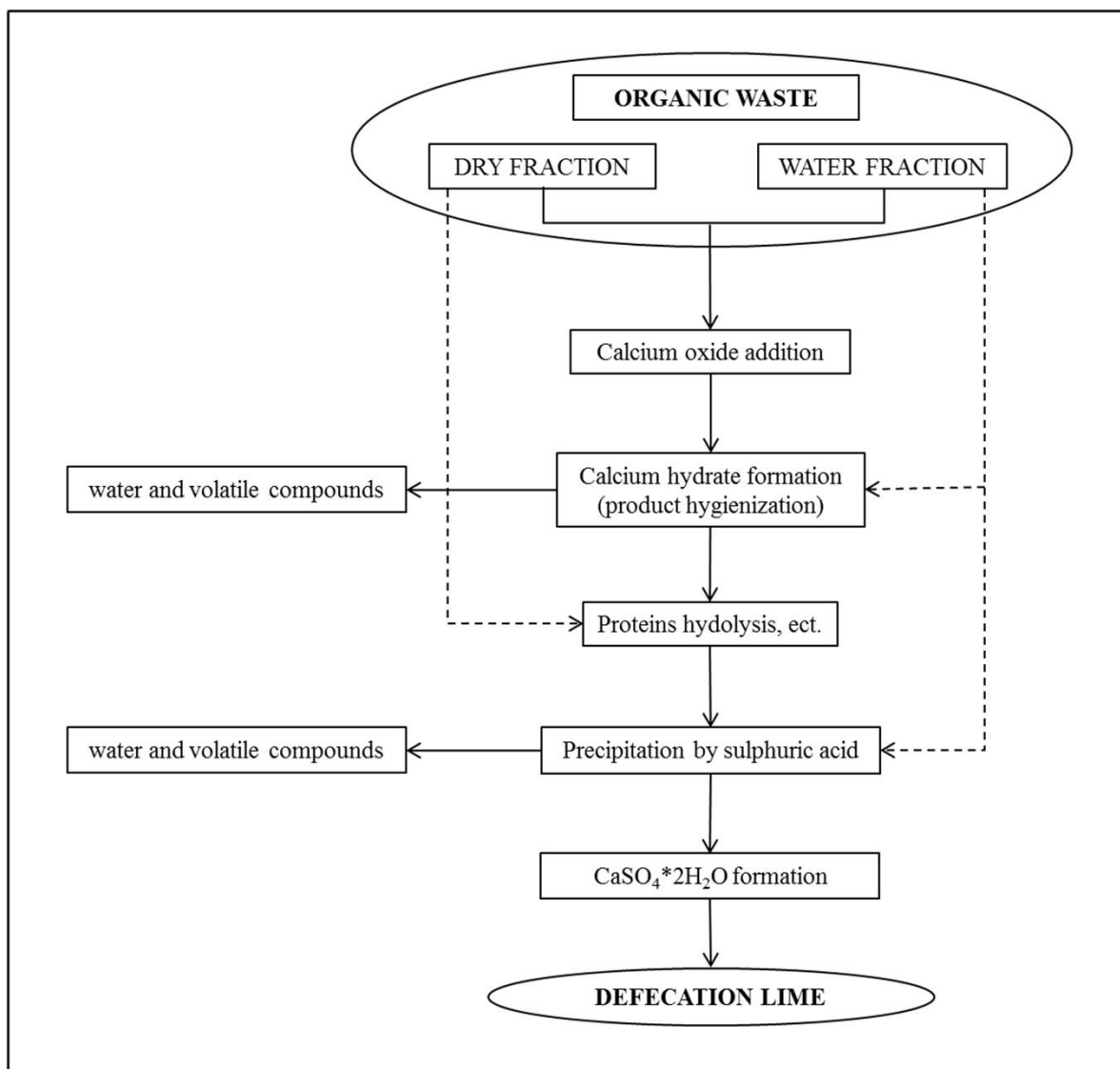
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492 **Figure 2.** Pipeline of the defecation lime production according to Alan srl (Italy).



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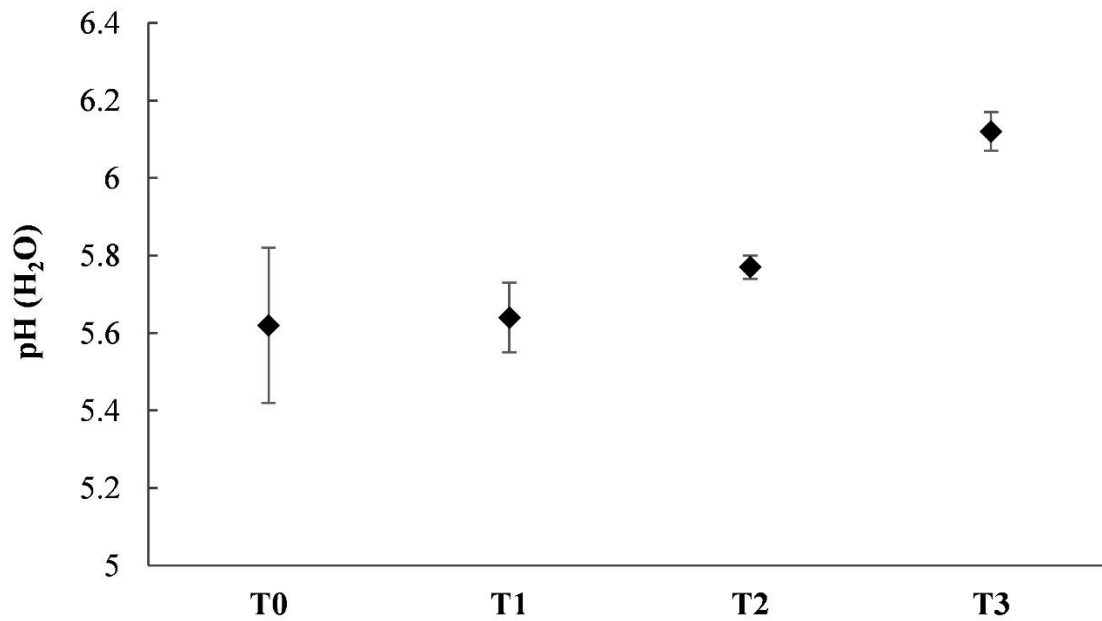
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503 **Figure 3.** pH values observed at the end of the two-year field experiment. T0=unfertilised,
504 T1=common farmer paddy field management including pre-sowing organic fertilisation (hoof
505 and horn) + chemical fertiliser, T2=defecation lime + chemical fertiliser, T3=defecation lime
506 at pre-sowing. Bars are \pm mean standard error.



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