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1 **A highly-sensitive microplate fluorimetric method for the high-throughput**  
2 **determination of nitrate ion in aqueous compost extracts.**

3  
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8  
9 **Abstract**

10 In this paper, a new spectrofluorimetric method for the determination of nitrate in aqueous  
11 compost extracts is presented. The microplate procedure is based on the reduction of nitrate  
12 to ammonium by means of Zn powder under acidic conditions and the following derivatization  
13 of ammonium with o-phthalaldehyde (OPA) and N-acetylcysteine (NAC) to give a fluorescent  
14 derivative. Optimization work allowed performing the reduction in 30 min on a small sample  
15 volume (150  $\mu\text{L}$ ) and using a little quantity of metal (20 mg). The use of strong acids is also  
16 avoided. Excellent limit of detection was achieved ( $1.3 \mu\text{M} = 0.08 \text{ mg}\cdot\text{L}^{-1} \text{ NO}_3^-$ ) along with good  
17 precision levels (4.2 and 14.3 % for 50 and 5  $\mu\text{M}$ , respectively) and a satisfactory linear  
18 dynamic range (4 - 100  $\mu\text{M}$ ). A good agreement between data coming from our method and  
19 from ion-exchange chromatography was found on real compost samples, indicating a good  
20 level of accuracy.

21  
22  
23 **Keywords:** Nitrate, microplate, compost extracts, spectrofluorimetric.  
24  
25

26 **1. Introduction**

27 Composting represents an useful way to recycle organic wastes into a fertilizing product. The  
28 formation of compost occurs by means of an exothermic and aerobic process promoted by  
29 micro-organisms who decompose the organic matter. The physical and chemical changes that  
30 occur during the compost formation determine the loss of the putrescibility of the biomass  
31 which also undergoes a partial mineralization [1-3]. The industrial importance of compost lies  
32 in its capability to enhance the agronomic quality of soil. Its employment in the agricultural  
33 industry has proved to be able to increase the yield of agronomic and horticultural crops and  
34 to restore soil conditions after tree harvesting [4]. Moreover, it represents a valid alternative to

35 the traditional incineration and to landfills. In fact, thanks to the water loss and to the  
36 mineralization of organic matter, the global volume and mass of the initial waste products can  
37 be reduced up to 50% [5, 6].

38 The monitoring of the composting process is essential not only to pursue a correct waste  
39 management but also to preserve the economic value of a commercial item. During the  
40 composting process nitrogen present in the biomass goes through two main mineralization  
41 phenomena: ammonification (production of  $\text{NH}_4^+$ ) and nitrification (production of  $\text{NO}_3^-$ ).  
42 Nitrogen-based parameters (i.e. concentration of ion nitrate and ammonium, and  $\text{NH}_4^+/\text{NO}_3^-$   
43 ratio) have proved to be useful to monitor the correct development of the composting process.  
44 In fact, while ammonium concentration tends to decrease during the compost stabilization,  
45 formation of nitrate is facilitated in mature compost thanks to the lower respiration rates [7].  
46 Monitoring of nitrate in various environmental samples in the frame of the 1991 European  
47 Union Nitrates Directive [8] (which aims to protect water quality across Europe by preventing  
48 nitrates from agricultural sources polluting ground and surface waters) can also benefit from  
49 efficient analytical techniques applied to aqueous compost extracts. For these reasons, the  
50 attention of researchers and enterprises has been focused on the assessment of reliable  
51 analytical protocols aimed to quantify these chemical species in compost and compost  
52 extracts. In the past, various methods have been proposed for the quantification of nitrate ion  
53 in aqueous solutions including spectrophotometry [9, 10], fluorimetry [11, 12], ion  
54 chromatography [13] and flow injection analysis [14]. Literature also offers some examples of  
55 microplate-based procedures where nitrate is first reduced to nitrite which is later  
56 spectrophotometrically revealed after derivatization [15, 16]. In spite of the strong contribution  
57 given by these works for the determination of nitrate, the main problem related to the cited  
58 procedures is given by the use of cadmium, which is renowned for his high toxicity, and also  
59 by the strong interferences caused by complex samples such as compost extracts, when the  
60 measurement is carried out by spectrophotometry.

61 The main goal of our work was to develop a new sensitive method for the determination of  
62 nitrate by a microplate-based procedure in compost aqueous extracts. In the past, our research  
63 group has developed various analytical methods based on the 96-wells technique [17-20]. In  
64 particular, a fluorescence-based microplate procedure for the quantification of ammonium and  
65 primary amines in compost water extracts and other matrices was recently assessed [17]. The  
66 basic idea for the assessment of our method was to exploit the previous protocol, reducing first  
67 the nitrate to ammonium and then quantifying the latter by means of the method already  
68 assessed. An important advantage of this analytical strategy would be the easy determination  
69 of both nitrate and ammonium ions with a single set of detection reagents (with or without the  
70 reduction step), which would be highly valuable as the  $\text{NH}_4^+/\text{NO}_3^-$  ratio is one of the principal  
71 nitrogen-based indicators of compost maturity [7].

72 In the last years, metals have been employed for the reduction of nitrate to nitrite or ammonia  
73 in aqueous solutions. Reducing properties of cadmium, aluminum, zinc, iron and also of some  
74 types of alloys like Devarda's alloy and Arndt's alloy have been reviewed by Fanning [21].  
75 Among all, Zn seems to represent a good candidate for the reduction of nitrate to ammonium.  
76 Indeed, besides being less toxic than other metals (i.e. cadmium), it has been already  
77 successfully used under mild reduction conditions for the quantification of nitrate in water  
78 solutions and other matrices [22-24], but never for compost extracts so far. For this reason, we  
79 present in this work a new fluorescence-based microplate method for the quantification of  
80 nitrate in compost water extracts which includes the conversion to ammonium by means of Zn  
81 powder, and which is developed as a simple analytical tool that can be used directly in  
82 composting platforms in a non-specialized environment (no fume hoods for strong acids and  
83 non-expert technicians).

84

## 85 **2. Experimental**

86

### 87 ***2.1 Samples and water extraction of organic matter***

88 Two type of compost samples were selected for this study. More specifically, the first type of  
89 samples (A samples) was exclusively composed by green wastes deriving from activities of  
90 gardening and maintenance of green public or private areas. Sampling sites were located in 3  
91 private areas (agricultural lands) located in Provence Alpes-Côte d'Azur region (France, GPS  
92 coordinates : near Gardanne : 43.466350N, 5.454541E ; near Rousset : 43.468935N,  
93 5.634117E, and near Rougiers : 43.411657N, 5.841994E). Ages of the swaths were always  
94 included between one week and twelve months. It should be noticed that studied swaths can  
95 be very heterogeneous in their composition of plant species, size and height. The second type  
96 of samples (B samples) consisted in sewage sludge mixed with green wastes (1/3-2/3, v/v). In  
97 this case, samplings were performed on an industrial composting platform (Biotechna) located  
98 in Ensûès-la-Redonne (Bouches-du-Rhône, France, GPS coordinates 43.376838N,  
99 5.190849E), A single swath was studied in a period of time included between 1 week and 6  
100 months, with a various frequency of sampling (samplings were performed every week during  
101 the 2 first months, every two weeks the next month, and every month the three months  
102 afterwards). For each type of compost, matter was collected in different places of the swath at  
103 80 cm depth, in order to get a final composite sample of 2 kg representative of compost.

104 The water extractions were performed in PTFE Teflon tube (triplicates per soil sample) on an  
105 orbital shaker (Fisher Scientific Bioblock SM30B) at 125 rpm and at room temperature. Organic  
106 matter was extracted through shaking 6 g of fresh compost with 60 mL of ultrapure water  
107 (resistivity of 18 M $\Omega$  cm<sup>-1</sup> at 25 °C, and total organic carbon < 20  $\mu$ g L<sup>-1</sup> ) with a soil/water ratio  
108 of 1/2 (w/v) for 2 h under N<sub>2</sub>. After extraction step, the tubes were centrifuged at 8000 rpm for

109 15 min (JP SELECTA, Medifriger BL-S), and the supernatants were collected and filtered  
110 through 0.45 µm membrane filters (PES membrane, Millipore, France). The resulting solutions  
111 were stored in the dark at -18 °C until analysis.

112

## 113 **2.2 Reagents and solutions**

114 All chemicals were of analytical reagent grade and used without further purification. Zn powder  
115 (100 mesh) and Zn granules were purchased from Alfa Aesar (Germany) and Prolabo  
116 (France), respectively. Copper granules were purchased by Fluka (France) while Fe powder  
117 (puriss., <212 µM) and Al powder were provided by Sigma-Aldrich (France). O-phthaldehyde  
118 (OPA) was obtained from Acros Organics (Belgium) and N-acetyl-L-cysteine (NAC) from  
119 Sigma-Aldrich. Buffers solutions were prepared dissolving the proper amount of sodium  
120 acetate (Sigma-Aldrich), 4-(2-Hydroxyethyl)piperazine-1-ethanesulfonic acid (HEPES, Acros  
121 organics), anhydrous sodium carbonate (Sigma-Aldrich) and sodium tetraborate decahydrate  
122 (Sigma-Aldrich) in ultrapure water (Millipore, USA, resistivity >18 MΩ cm) and adjusting the pH  
123 with sodium hydroxyde or hydrochloridic acid.

124 Stock standard nitrate solution (0.1 M) was prepared by dissolving appropriate amount of  
125 sodium nitrate (Sigma-Aldrich) in ultrapure water. Working solutions were obtained by diluting  
126 stock solutions to proper concentrations.

127

## 128 **2.3 Instruments**

129

### 130 **2.3.1 Microplate**

131 Microplate fluorescence measurements were carried out on a microplate reader (Infinite M200,  
132 Tecan France SAS, France), operating at 30 °C and controlled by i-control™ software (Tecan).  
133 Detection was performed by top fluorescence reading at  $\lambda_{\text{ex}} = 415 \text{ nm}$  and  $\lambda_{\text{em}} = 485 \text{ nm}$ . Other  
134 parameters were as follows: gain: 80; number of flashes: 5; integration time: 20 µs.  
135 Fluorescence intensities were expressed in arbitrary units (a.u.). Polystyrene black 96 V-well  
136 microplates (Fisher Scientific, France) were used.

137

### 138 **2.3.2 Ion chromatography analysis of nitrate**

139 Nitrate analysis was carried out by ion chromatography (EN ISO 10304-2:1996) on a ICS-3000  
140 HPLC system (Dionex, USA), driven by Chromeleon® (6.80 version) equipped with a guard  
141 column (Dionex AG11-HC), an analytic Dionex AS11-HC (4 × 250 mm) column, a  
142 conductimetric detector (Dionex CD-25) and using a 200 µL loop injection valve. Analysis were  
143 performed in an isocratic mode (22.5 mM NaOH in helium sparged deionized water) at 30 °C,  
144 with a flow rate set at 1.5 mL min<sup>-1</sup>. To improve the signal-to-noise ratio of the conductivity

145 measurement, an external flow electrochemical suppressor system (ACRS 500 4 mm) was  
146 added to the analytical system.

147

#### 148 **2.4 Analytical protocol for nitrate determination**

149 Twenty mg of Zn powder (100 mesh) were dispensed into the wells of the microplate, 150  $\mu\text{L}$   
150 of sample or standard solution were introduced and 20  $\mu\text{L}$  of MES buffer (0.1 M, pH 6) were  
151 added. The plate was shaken for 30 min at 30 °C. Then, 120  $\mu\text{L}$  of the solution were transferred  
152 into new wells where 20  $\mu\text{L}$  of a solution of 20 mM NAC and 30  $\mu\text{L}$  of 13 mM OPA in ethanol-  
153 0.15 M carbonate buffer pH 10.5 (10:90, v/v) were subsequently added. The plate was shaken  
154 for 10 min and fluorescence intensity was then recorded, with excitation and emission  
155 wavelengths set at  $\lambda_{\text{ex}}=415 \text{ nm}/\lambda_{\text{em}}=485 \text{ nm}$ . Concentrations in unknown samples were  
156 determined using the linear calibration curves obtained with standards. Nitrate concentration  
157 was calculated after quantifying the ammonium by means of the microplate procedure  
158 previously assessed by our research group [17].

159

### 160 **3. Results**

161

#### 162 **3.1 Choice of the metal system for the reduction of nitrates**

163 The proposed analytical procedure is based on the reduction of nitrate ion to ammonium and  
164 the subsequent determination of the latter by fluorescence (Figure 1). One of the main  
165 challenges of this work was to find a metal for the effective reduction of nitrates under mild  
166 conditions (moderate temperature to apply the developed protocol in polystyrene microplates  
167 which don't withstand high temperatures, and also because the most affordable microplate  
168 readers don't enable heating above 40°C), but which does not interfere with the second step  
169 of the protocol (derivatization of ammonium with OPA/NAC and formation of isoindol adduct).  
170 Alloys like Devarda's alloy generally provide efficient reduction of nitrates, but only under quite  
171 harsh reaction conditions (i.e. boiling water). Several tests were performed in order to reduce  
172 the nitrate to ammonium with various metals. Experiments conducted with aluminum (foil and  
173 powder) and copper (granules) at various pH conditions did not provide any significant result  
174 proving to be not efficient for the reduction of the analyte. On the other hand, tests performed  
175 with iron (thread and powder) revealed that this metal is able to convert nitrate to ammonium  
176 in acidic conditions (encouraging results were obtained at pH 5 and 6). Unfortunately, the  
177 corrosion products formed during the reduction phase constituted a strong interference for the  
178 revelation of ammonium preventing a precise and accurate determination. Attempts to  
179 suppress the interference by adding EDTA at various concentration levels (0.1-1 M) were  
180 unsuccessful. Finally, Zinc proved to be promising both for reduction of nitrate and ammonium

181 derivatization under mild conditions in our first experiments, so we decided to optimize our  
182 method with this metal.

183

### 184 **3.2 Optimization of the analytical method with Zn**

185 Reduction of nitrate with Zn was performed in presence of various selected buffers in order to  
186 identify the best pH conditions for the conversion of nitrate to ammonium and for the  
187 derivatization. These first experiments were performed by means of Zn granules. A quantity of  
188 metal granules large enough to entirely cover the bottom of the microplate wells was cut and  
189 introduced. 100  $\mu$ L of a water solution of  $\text{NaNO}_3$  100  $\mu$ M were introduced in the wells containing  
190 the metal along with 20  $\mu$ L of : i) acetate buffer (0.1 M, pH 5), ii) MES buffer (0.1 M, pH 6), iii)  
191 HEPES buffer (0.1 M, pH 7), iv) carbonate buffer (150 mM, pH 10.5) , v) borate buffer (100  
192 mM, pH 11.5). Also HCl 2.5 M and  $\text{H}_2\text{SO}_4$  2.5 M were tested for the reduction. In this case,  
193 NaOH 0.1 M was added to the wells until basic pH, before derivatization with NAC and OPA.  
194 The suspensions were shaken for 1 h at 30 °C before addition of OPA and NAC reagents.  
195 Experiments were also conducted on a solution of  $\text{NH}_4\text{Cl}$  100  $\mu$ M in order to detect possible  
196 interferences of the reducing conditions on the ammonium derivatization. As shown in Figure  
197 2, best results were obtained at pH 5 and pH 6. In fact, in both cases conversion of nitrate to  
198 ammonium occurred and high values of fluorescence for ammonium were detected, resulting  
199 in good sensitivity under these conditions. According to data obtained, reduction of nitrate is  
200 much less efficient at pH 7 or when basic carbonate and borate buffers are employed, also  
201 probably because of the evaporation of  $\text{NH}_3$  which is predominant at neutral/basic pH.  
202 Regarding the acidic conditions selected for the study, it appears that in the case of HCl 2.5 M  
203 there is no substantial difference between the blank values and those of nitrate and  
204 ammonium, while in the case of  $\text{H}_2\text{SO}_4$  data related to ammonium derivatization are very low.  
205 The MES buffer (0.1 M, pH 6) was selected for the following experiments, given the higher  
206 value provided by the test on the ammonium solution revealing a higher sensitivity for this pH  
207 level. The subsequent experiments were mainly aimed to optimise the conditions for the  
208 enhancement of the conversion of nitrate to ammonium.

209 A kinetic test was carried out in order to select the best time condition for the reduction of  
210 nitrate with zinc powder (more efficient than zinc granules). As shown in Figure 3, after 30 min  
211 about 90% of the nitrate is reduced to ammonium indicating that this could represent a good  
212 time condition for the proposed procedure. The influence of Zn powder quantity on the  
213 reduction rate at the selected conditions (30 °C, 30 min) was also studied. Calibration curves  
214 obtained with 10, 20 and 30 mg of Zn are shown in Figure 4. Tests performed with 10 mg of  
215 powder showed that there is a loss of linearity at concentrations higher than 50  $\mu$ M indicating  
216 also that, in this case, contact surface is not large enough to provide efficient conversion of  
217 nitrate to ammonium. At the same time, experiments carried out with 30 mg of powder, showed

218 a worsening of the  $R^2$  value probably due to an interference caused by the excess of solid  
219 particles in the suspension. For this reason, 20 mg was selected as the best compromise for  
220 the procedure.

221 Finally, influence of an increase of the reaction temperature was also assessed (up to 40 °C,  
222 the maximum heating capacity of most microplate readers/shakers), with no significant  
223 improvements, and 30 °C was therefore selected for the optimized protocol.

224

### 225 **3.3 Interferences**

226 The interference of various major anions and cations that could be found in compost extract  
227 samples was investigated. More specifically,  $K_2SO_4$ ,  $KH_2PO_4$ ,  $Na_2CO_3$ ,  $MgCl_2$ ,  $NaCl$ ,  $NaBr$  and  
228  $CaCl_2$  were selected for the study. Other less common redox active anions (low redox  
229 potential/high reducing ability) such as  $HPO_3^{2-}$ ,  $SO_3^{2-}$  and  $C_2O_4^{2-}$  have also been included in  
230 these interferences tests. The analytical protocol was applied to pure solutions of the cited  
231 salts or anions at a concentration of 2 mM, giving a response not significantly different from  
232 the blank. Moreover, tests were also performed on mixtures of  $NaNO_3$  100  $\mu M$  and selected  
233 interfering compounds in order to compare results with those given by the pure  $NaNO_3$ . Results  
234 showed that the salts tested did not interfere up to at least 2 mM.

235 Experiments conducted on a standard solution of  $NaNO_2$  100  $\mu M$  revealed that nitrite shows  
236 the same behavior as nitrate, being reduced to ammonium by Zn at the same pH conditions.  
237 Anyway, analysis performed by ion-exchange chromatography on the samples confirmed the  
238 already expected absence of nitrite in the aqueous compost extracts. Interferences from nitrite  
239 should therefore be negligible in this type of environmental samples, but has to be considered  
240 (separate quantification by Griess reagent) if the method is applied to other samples with  
241 significant nitrite concentrations.

242

### 243 **3.4 Analytical features**

244 The assessed procedure was validated in terms of sensitivity, linearity, precision and accuracy.  
245 The limit of detection (LOD) was calculated from the residual standard deviation of the  
246 regression (linearity study method). More specifically, LOD was evaluated as follows:

$$247 \text{ LOD} = 3s/b$$

248 where  $s$  is the standard deviation (SD) of the a-intercept and  $b$  the slope of the calibration  
249 curve. LOD is 1.30  $\mu M$  (0.08  $mg L^{-1}$ ). It is interesting to note that detection limit is almost an  
250 order of magnitude smaller than the LOD estimated by Murray et al. [22], for their procedure  
251 using zinc reduction and a spectrophotometric method (0.5  $mg L^{-1}$ ).

252 The calibration curve ( $I = 11.10C + 316.2$ ;  $I$  = fluorescence intensity (arbitrary units),  $C$  = nitrate  
253 concentration ( $\mu M$ )) was linear up to 100  $\mu M$  with a correlation coefficient of 0.9941, resulting  
254 in a large enough linear dynamic range for our analytical purposes (4.5-100  $\mu M$ ). Relative

255 standard deviations were assessed on a 5  $\mu\text{M}$  (RSD = 14.3%) and on a 50  $\mu\text{M}$  (RSD=4.2%)  
256 nitrate standards (n=10 replicates) indicating a good repeatability for both concentration levels.

257

### 258 **3.5 Comparison with other microplate methods for nitrate determination**

259 The microplate format is an ideal tool for routine analysis when a large number of samples has  
260 to be analyzed in a short time (typical case for ammonium or nitrate determination in aqueous  
261 samples). All methods adapted in microplate are based on the reduction of nitrate to nitrite and  
262 subsequent spectrophotometric measurements after reaction with Griess reagent. Reduction  
263 of nitrate to nitrite has been performed by various methods: copperised cadmium granules [25]  
264 or pins [15], vanadium (III) [26] or enzymatic reduction [27]. Global reaction times are a bit  
265 longer with these methods (60-90 min at room temperature) compared to our method (40 min).  
266 The main advantage of these spectrophotometric techniques is the low detection limits (12-20  
267  $\mu\text{g L}^{-1}$ ) which are 4 to 6 times lower than our spectrofluorimetric method. However, these  
268 methods also have limitations for compost analysis: i) the main reduction methods still use  
269 cadmium which is highly toxic; ii) complex and colored samples such as compost extracts will  
270 display strong matrix effects using spectrophotometric techniques [28], making fluorimetric  
271 measurements highly desirable; iii) determination of nitrate and ammonium ( $\text{NH}_4^+/\text{NO}_3^-$  ratio is  
272 one of the principal nitrogen-based indicators of compost maturity) requires two sets of  
273 detection reagents if the Griess reagent is used, while our methodology easily provides  
274 quantification for both analytes (with or without reduction step).

275 Similarly, nitrate determination using nitration of resorcinol and spectrophotometric  
276 measurement is not suitable for this type of analysis, as it uses pure sulfuric acid (not  
277 compatible with microplates) and displays strong interferences due to sample color (own  
278 experiments, data not shown).

279 Overall, our fluorimetric method seems more adapted to compost extract analysis in the  
280 microplate format than methods based on spectrophotometry.

281

### 282 **3.6 Quantification of nitrate ion and method validation in aqueous compost extracts**

283 Nitrate was quantified by means of the assessed procedure in twenty-five aqueous extracts of  
284 compost of various sources, using the external calibration method. Sample dilution represents  
285 a critical point of the whole analytical protocol because the highly-colored matrix can heavily  
286 disturb spectroscopic measurements. Moreover, dilution is required in order to match the  
287 defined calibration range both for ammonium and nitrate. A comparison between the slope of  
288 calibration curves obtained by means of external calibration and multiple standard addition  
289 methods for some selected samples was exploited in order to select a dilution ratio able to  
290 minimize the interferences given by matrix, but still able to detect nitrates and ammonium. A

291 5-fold dilution was adopted for A samples and a 50-fold one for B samples. Nitrate  
292 concentration was quantified in a range included between  $2.0\pm 0.1$  and  $90\pm 10$  mg L<sup>-1</sup>.  
293 These twenty-five samples were also chosen for comparison purposes between ion-exchange  
294 chromatography and microplate measurements with the developed procedure. As shown by  
295 Figure 5, a good correlation ( $R^2=0.9127$ ) was found between the two data sets, and a very  
296 weak intercept value with regard to the studied concentration range sets. The slope reveals a  
297 slight underestimation of data obtained by the microplate method, probably due to residual  
298 matrix effects of these compost samples. A higher dilution rate would have improved these  
299 results, but was not possible because most samples would have too low concentrations for  
300 proper nitrate or ammonium quantification. Results are still satisfactory for a field test tool,  
301 which is aimed to assess the evolution of nitrate concentration during the composting process  
302 and which mainly requires a good correlation between the measurements and the actual  
303 concentrations in the compost samples.

304

#### 305 **4. Conclusions**

306 In this study, we presented the assessment and validation of a new analytical method using  
307 the microplate format for the determination of nitrate ion in aqueous compost extracts.  
308 Optimization work allowed to obtain fast and stable responses by employing a small quantity  
309 of a non-toxic metal (zinc) for the reduction. Nitrate reduction with zinc (mainly studied for the  
310 reduction of nitrate to nitrite, much less to the final ammonium reduction), was coupled with a  
311 fluorimetric detection method, offering several advantages such as lower detection limits (an  
312 order of magnitude lower than the spectrophotometric method described with zinc reduction  
313 [22], especially using the microplate format) and less interferences due to complex and colored  
314 samples such as compost extracts. It also potentially enables the easy determination of both  
315 nitrate and ammonium ions with a single set of detection reagents (with or without the reduction  
316 step), which is highly valuable as the  $\text{NH}_4^+/\text{NO}_3^-$  ratio is one of the principal nitrogen-based  
317 indicators of compost maturity. Besides, no strong acids are used in this method, on the  
318 contrary of other frequently used analytical methods for nitrate [10,12]. Preventing the use of  
319 strong acids or toxic metals and reagents is highly desirable for this type of analytical method  
320 which is meant to be used directly on composting platforms by non-expert technicians. Only a  
321 small volume of sample is required for the analysis and the whole procedure is completed in  
322 about 40 min with the possibility to analyze several extracts at the same time thanks to the  
323 microplate instrumentation. Validation showed that the proposed procedure is sensitive,  
324 precise and with a good level of accuracy. For all these reasons, the method is a good  
325 candidate for routine analysis of nitrate in aqueous compost extracts, either in a traditional  
326 laboratory or directly in composting platforms. A simple on-site compost extraction with a

327 modified coffee machine is currently under evaluation in our laboratory in order to reach a full  
328 on-site analytical procedure.

329

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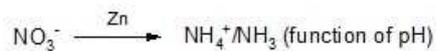
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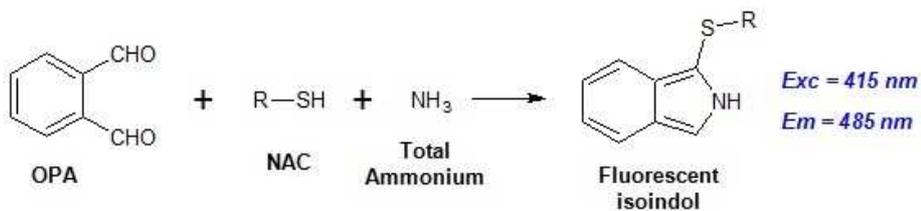
### Figure captions

**Figure 1.** Proposed protocol for nitrate determination; **Figure 2.** Influence of the pH of the reduction step on the fluorescence intensity response of standard solutions after reduction and derivatization with OPA/NAC (zinc granules, 1h reduction time, n=2). **Figure 3.** Influence of the time of the reduction step on the fluorescence intensity response of standard solutions after reduction and derivatization with OPA/NAC (20 mg zinc powder, n=2). **Figure 4.** Calibration curves (0-100 µM) depending on the quantity of zinc powder used for the reduction step (30 min reduction time, n=2). **Figure 5.** Comparison between microplate method and ion-exchange chromatography (n=2).

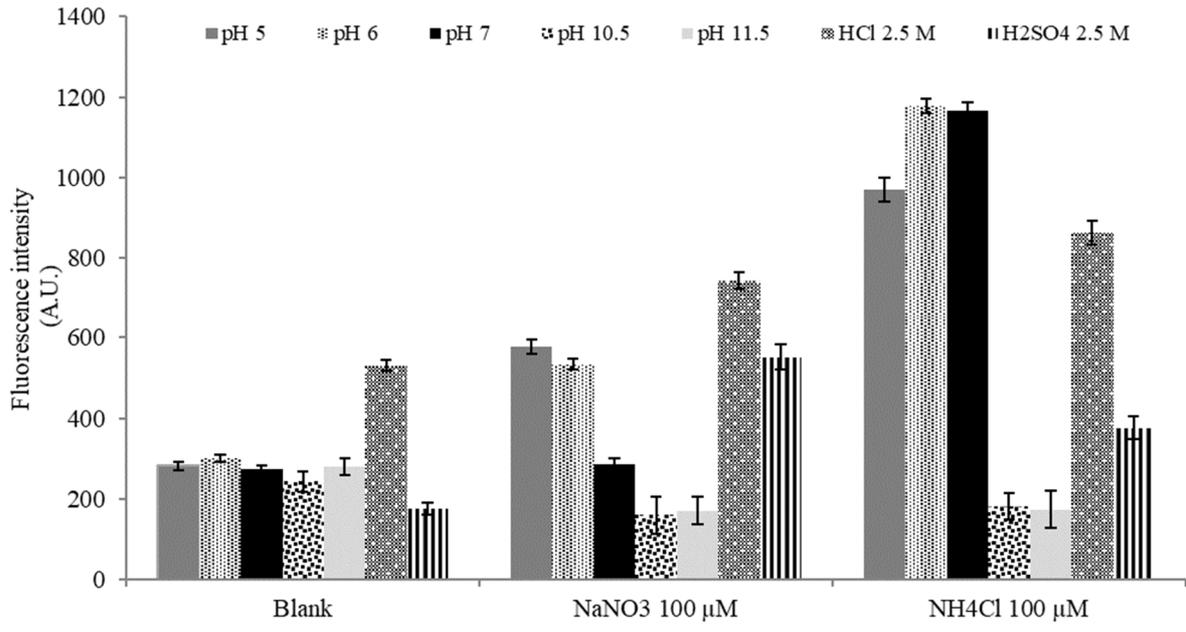
Step 1: reduction



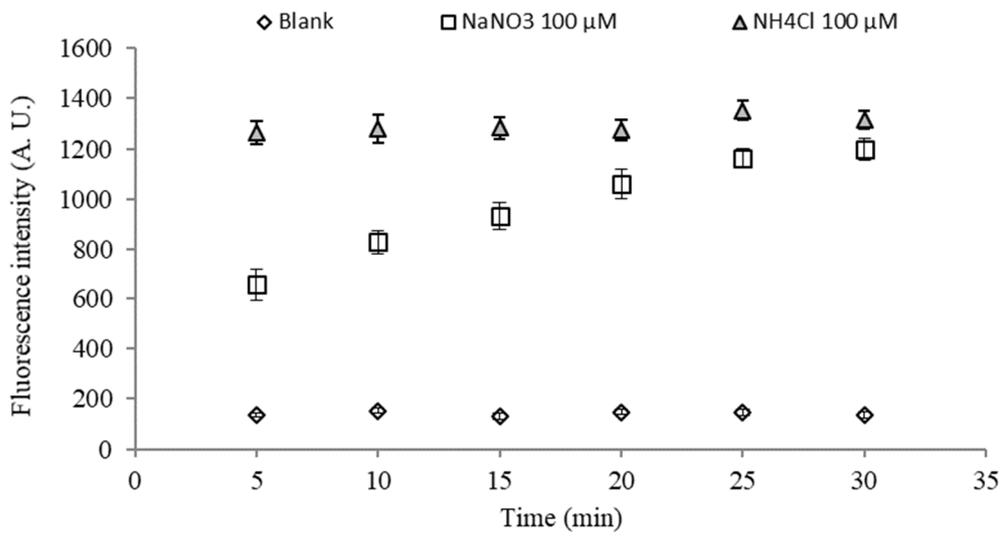
Step 2: derivatization



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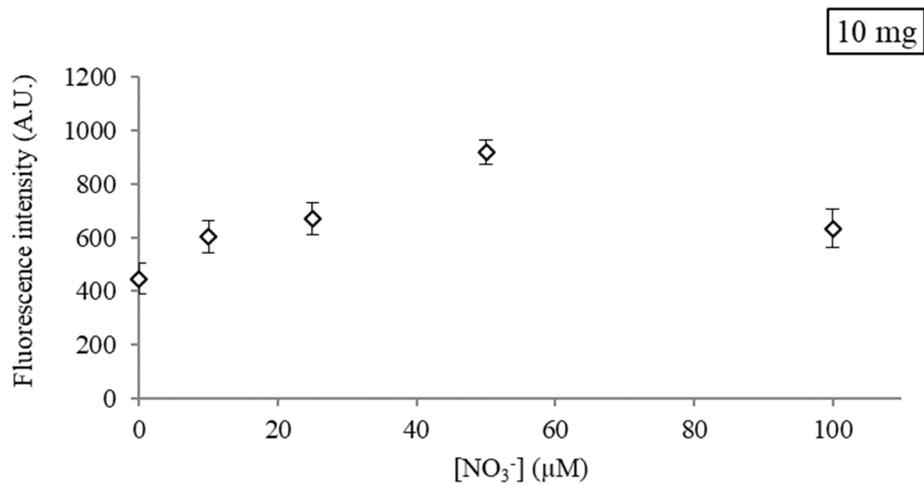


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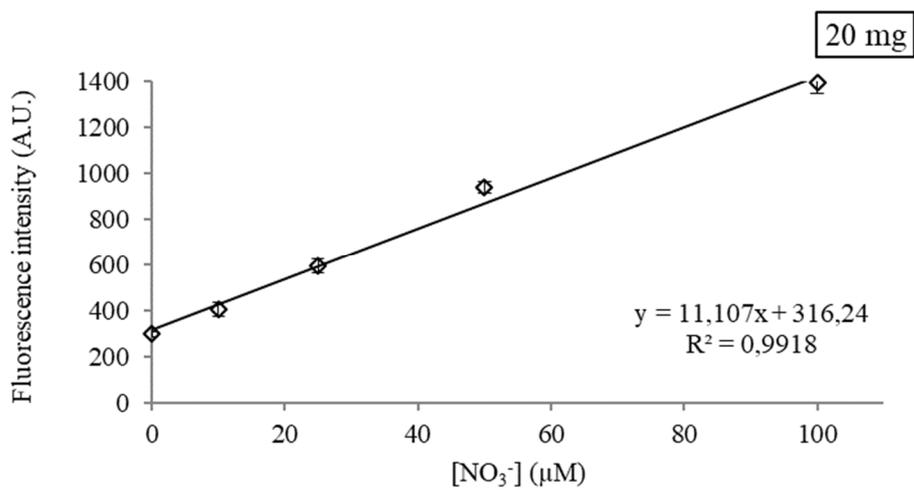


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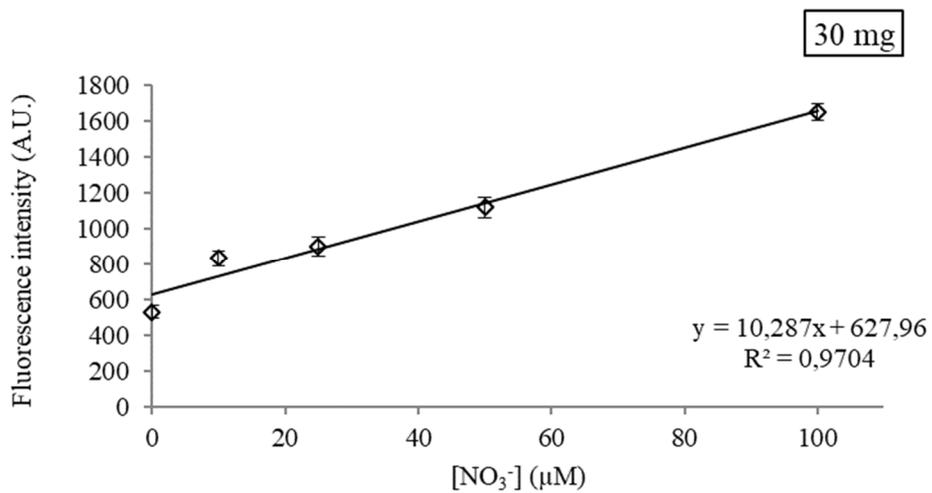
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