

# Comparison Between Sensing Systems for Ammonium Detection And Measurement In Soil

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**Abstract.** Usually, ammonium in soil is carried out through steam distillation of ammonia obtained after alkalization of soil extracts and further back titration of the collected solutions. Alternatively, ion selective electrodes (ISE) specific for ammonium ions can be used, in order to measure their concentration in aqueous soil extracts. The aim of this study is to assess the possibility to use, alternatively to the previous techniques, two kinds of chemical sensors able to measure  $\text{NH}_3$ , such as an interdigital microelectrode (IDE) coated of conductive polymer and a sensors array, usually named electronic nose (EN), based on quartz crystal microbalances (QCMs) covered with functionalized polymers. These sensors were chosen on the base of their ability to detect  $\text{NH}_3$  in sample headspace (specifically or aspecifically, respectively). Therefore,  $\text{NH}_4^+$  in solution was converted to  $\text{NH}_3$  by alkalizing soil extracts. Sensors were calibrated at first against known concentrations of  $\text{NH}_4^+$ . Results were compared with those obtained with an ISE for  $\text{NH}_4^+$ .

**Keywords:** Electronic nose; interdigital sensors; ion selective electrodes; soil; ammonium; ammonia.

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## INTRODUCTION

Nitrogen (N) is an essential nutrient for all organisms. In soil, N can be found in several forms, both organic and inorganic. The main inorganic forms of N in soil are  $\text{NH}_4^+$ ,  $\text{NO}_3^-$  and  $\text{NO}_2^-$ . The importance of these forms, specifically  $\text{NH}_4^+$  and  $\text{NO}_3^-$ , lays in their being the main and more direct forms absorbed by organisms. Nitrate and nitrite are highly soluble in soil and then more bioavailable but also more leachable, since as anion they are repulsed from negatively charged soil colloid surface. On the contrary,  $\text{NH}_4^+$  can be highly adsorbed onto soil particles, owing to its positive charge, and it can also be entrapped into soil minerals. To measure the bioavailable  $\text{NH}_4^+$  in soil, it must be previously desorbed from soil particles, by extraction with neutral salts solutions ( $\text{KCl}$  or  $\text{K}_2\text{SO}_4$ ) [1], able to exchange the adsorbed  $\text{NH}_4^+$  with  $\text{K}^+$ . Extracts can then be measured in their  $\text{NH}_4^+$  content using different procedures. These comprise colorimetric methods, titration methods and the use of ISEs [1]. Currently, colorimetric methods are used after  $\text{NH}_4^+$  extraction and its conversion to  $\text{NH}_3$  in strong alkaline solutions ( $\text{NaOH}$ ) and reaction with different reagents to form a chromophore (indophenol blue method [1], Nessler method [1], salicylate method [2]). Differently,  $\text{NH}_4^+$  can be converted to  $\text{NH}_3$  in strong or mild alkaline solutions ( $\text{NaOH}$  or  $\text{K}_2\text{CO}_3$ , respectively), which after diffusion into the atmosphere is collected in a boric acid solution and then titrated with  $\text{H}_2\text{SO}_4$  or

determined colorimetrically (steam distillation method [1, 2] and microdiffusion method [1], respectively). Alternatively to these methods, ISEs can be used to measure  $\text{NH}_4^+$  in soil extracts. Relative to the previous methods, the use of ISEs is easier to use and not time consuming. Two kinds of ISEs are commercially available, which can measure either  $\text{NH}_4^+$  or  $\text{NH}_3$ . In this latter case, a previous conversion of  $\text{NH}_4^+$  to  $\text{NH}_3$  by alkali addition is required. However, in all the methods where strong alkali (e.g.  $\text{NaOH}$ ) is added directly to soil extracts, it is probable that alkali-labile organic N compounds are hydrolysed and that they release additional  $\text{NH}_4^+$ , then overestimating the measures [1]. For this reason an ISE for  $\text{NH}_4^+$  was preferred as reference method.

Interdigital sensors are well suited to quantitative and qualitative analyses of gases and vapours. The surfaces of the sensors are usually coated with substances commonly adopted in gas chromatography. The signals for gas analysis are the changes in electrical conductance and capacity. IDEs are capable, in fact, to measure both the dielectric properties (capacitive transducers, IDCs) and the conductance (resistive transducers, IDEs) of the material placed both among their fingers and above. IDEs are used in telecommunications, biotechnology, chemical sensing, dielectric imaging, acoustic sensors, and microelectromechanical systems (MEMS) applications [3]. In the present study an IDE transducer coated with a polymer selective for ammonia was used.

Electronic noses (ENs) consist of a sensors array able to perceive volatile (VOCs) or semivolatile organic compounds (SVOCs) present in sample headspace, i.e. the "odour", and to transduce the consequent chemical or physical changes of the sensing films into electric signals. ENs have been involved in several applications: biomedicine [4], agroindustry and food quality [5], environmental and industrial analyses [6, 7]. Sensors in the arrays may be specific for single analytes, or for a chemical class of compounds or completely aspecific. The EN purposely set up in this study comprised both specific and aspecific polymers for  $\text{NH}_4^+$  detection.

The aim of this study was to compare the capacity of these three sensors, based on different principles potentiometry, chemoresistivity and gravimetry, to perceive and measure  $\text{NH}_4^+$  in soil.

## EXPERIMENTAL AND METHODS

### Samples Preparation

Soil used for detection of ammonium was a clay loam forest soil. Before extraction soil was treated with  $\text{NH}_4\text{Cl}$  at increasing concentrations (250 mM, 500 mM and 2 M) for 1 h under shaking, in order to saturate with  $\text{NH}_4^+$  all soil sites available for cation binding, and then to simulate soil with different  $\text{NH}_4^+$  concentrations. Then, soil was filtered on paper discs deprived of  $\text{NH}_4^+$ ; the recovered soil was then extracted with 2 M  $\text{KCl}$  (1:10 w/v) under shaking for 1 h, then filtered, and the solutions were tested for  $\text{NH}_4^+$ . Controls without previous  $\text{NH}_4^+$  saturation (native soil) were extracted with 2 M  $\text{KCl}$  only. All the measurements were carried out in a thermostated incubator (Stewart S160D) set at  $25 \pm 0.5^\circ\text{C}$ .

### Ion Selective Electrode

The measurements of  $\text{NH}_4^+$  concentrations in both standards and soil extracts were carried out by an ISE for  $\text{NH}_4^+$  (Crison) in beakers under stirring, and after adjusting ionic strength with 1 M  $\text{MgSO}_4$  (1:10 v/v) to uniform ionic strength in all samples. The potentials detected by ISE after contact with  $\text{NH}_4^+$  in soil extracts were referred to a reference curve, where  $\text{NH}_4^+$  concentrations (from 1  $\mu\text{M}$  to 1 M) in ultrapure water and their relative potentials were plotted in a logarithmic scale, in order to calculate the  $\text{NH}_4^+$  concentrations in the extracts. The measurements of  $\text{NH}_4^+$  were performed directly on soil extracts without any further treatment, if not specified. When the potential of soil extracts exceeded the maximal value in the calibration curve, soil extracts were diluted prior

to the adjustment of the ionic strength in order to have potentials within the range of the reference curve.

### Interdigital Microelectrodes

The IDE transducer had interdigital chromium electrode designed on oxidized silicon wafer (5640  $\mu\text{m}$  fingers length, 150  $\mu\text{m}$  fingers width and 150  $\mu\text{m}$  fingers spacing between them, respectively). IDE was coated of a nanostructured fibrous layer of doped polyaniline (polyaniline emeraldine base - PANi-EB), 10 kD molecular weight (Mw) and polyethylene oxide (PEO) 200 kD Mw, deposited by electrospun technique. After alkalization of soil extracts with  $\text{MgO}$  for 10 min under stirring of the various solutions placed in sealed flasks, dynamic measurements were carried out using a 4 channels MKS 247 mass flow controller, where  $\text{N}_2$  (carrier) was mixed with samples headspaces (5% v/v) prior to measurements. The concentrations of  $\text{NH}_4^+$  in the solutions were calculated by the reference of the electric resistance variations (Ohm), measured by IDE exposed to the atmosphere ( $\text{NH}_3$ ) of the same solutions, to a calibration curve obtained by plotting  $\text{NH}_4^+$  concentrations in ultrapure water (ranging from 50 nM to 5  $\mu\text{M}$ ) versus the electric resistance changes induced in IDE by exposition to their relative headspaces. Dilutions were carried out in samples exceeding the reference curve.

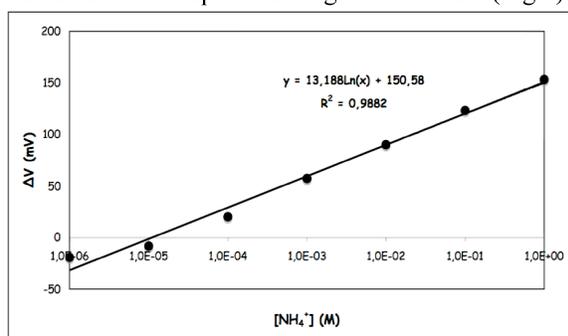
### Electronic Nose

The EN used in the present study was comprised of an array of 7 sensors consisting of QCMs coated with diverse organic films. Specifically, 5 polymers (polymethylphenylsiloxane, polyethylenimine, polyacrylonitrile, 6-polyamide, PANi-EB) with different physical and chemical features (polarity, basicity, hydrophobicity) and 2 different metalloporphyrins (Zn, Mn), deposited as thin films onto QCMs oscillating at 20 MHz, located in a 10 ml measuring chamber. The  $\text{NH}_4^+$  present in both soil extracts and standards placed into sealed flasks was measured after its conversion to  $\text{NH}_3$  similarly to what described for IDE sensors. EN measurements were carried out by diverting a 200 sccm  $\text{N}_2$  flux into the flasks through a 4 channels Mass Flow Controller MKS 247. Frequency variations of QCMs measured at equilibrium between gases or volatile analytes adsorbed onto coating films and those present in the chamber's atmosphere were processed through Principal Component Analysis (PCA). At first, a calibration curve was obtained by processing through PCA the variations in frequency (kHz) measured by EN when exposed to the headspaces of standard

solutions in ultrapure water containing known concentration of  $\text{NH}_4^+$  (ranging from 100  $\mu\text{M}$  to 5 mM) in order to obtain PC1 values, which were then plotted against  $\text{NH}_4^+$  concentration. Then, the variations in frequency (kHz) measured by EN exposed to headspaces of extracts with unknown  $\text{NH}_4^+$  contents were processed by using the same algorithm used to carry out the PCA of measures relative to standard solutions. Then, the reckoned PC1 values relative to soil extracts were used in the equation of the calibration curve to determine  $\text{NH}_4^+$  content of the extracts. Soil extracts were suitably diluted when relative PC1 values exceeded the reference curve.

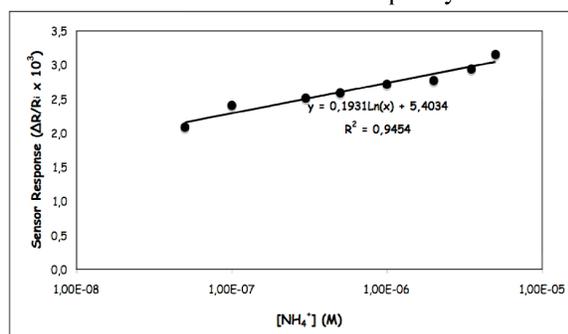
## RESULTS

The reference curve of  $\text{NH}_4^+$  concentrations vs. their relative potential measurements obtained by ISE showed a linear response on logarithmic scale (Fig.1).



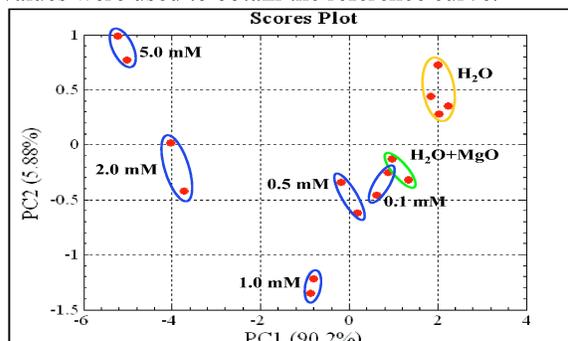
**FIGURE 1.** Calibration curve obtained by plotting different concentrations of  $\text{NH}_4^+$  against their relative potential differences measured by ISE

In the reference curve obtained by IDE a progressive linear increase in electric resistance of IDE was observed at increasing  $\text{NH}_4^+$  concentrations on a logarithmic scale (Figure 2). In measurements performed with EN, the relationship between  $\text{NH}_4^+$  concentrations and their relative frequency shifts was



**FIGURE 2.** Calibration curve obtained by plotting different concentrations of  $\text{NH}_4^+$  against their relative potential differences measured by IDE

obtained by processing at first the frequency shifts by PCA (Figure 3) and then fitting PC1 values (carrying 90.2% of the data variance) vs. increasing concentrations of  $\text{NH}_4^+$  standard solutions alkalized with MgO. Since PC1 is the axis of PCA plots containing the greatest variance and it also separates various concentrations of  $\text{NH}_4^+$  in different clusters, its values were used to obtain the reference curve.

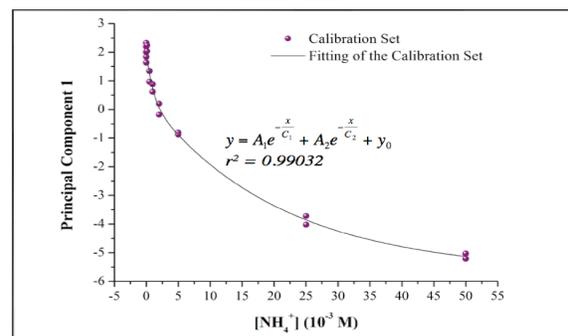


**FIGURE 3.** Scores Plot of the first two principal components as resulting from the calibration data set

The resulting relationship had a polynomial negative double exponential trend (Figure 4):

$$y = A_1 e^{-\frac{x}{C_1}} + A_2 e^{-\frac{x}{C_2}} + y_0; r^2 = 0.99032 \quad (1)$$

In the reference curves obtained by IDE and EN, lower  $\text{NH}_4^+$  concentrations ranges were used relative to those used in the calibration by ISE. This fact was due to the different (higher) sensitivity of the former sensors relative to the latter one.



**FIGURE 4.** Plot of the PC1 versus the ammonium concentration, as resulting from the PCA analysis (●). Data have been fitted to build a calibration curve (grey line).

Ammonium content measured by ISE in extracts from variously treated soils exceeded the calibration curve. Then extracts were diluted to include their  $\text{NH}_4^+$  contents in the reference curve. Since both IDE and EN sensors showed a higher sensitivity to  $\text{NH}_3$  derived from the conversion of  $\text{NH}_4^+$  in alkaline solution than that showed by ISE versus  $\text{NH}_4^+$ , soil

extracts were diluted much greatly than in ISE measurements.

Therefore, although all sensing systems tested in this study responded with increasing electric signals to increasing concentrations of  $\text{NH}_4^+$ , the range of  $\text{NH}_4^+$  concentrations practicable with different sensors was 1.5 order of magnitude lower with EN and even 4.5-5 orders lower with IDE than with ISE. This means that different sensors can be used for different purposes or in different experimental or environmental conditions. Therefore, in the presence of high  $\text{NH}_4^+$  concentrations, sensors with lower sensitivity (ISE) are recommended, while in opposite conditions a higher sensitivity is required ( $\text{EN} < \text{IDE}$ ). Nevertheless, if diluting is a straightforward practice to obtain samples which can be measured also with high sensitive sensors, concentrating the samples, to achieve values of the measured parameter within the reference curve, is a procedure which can have some controindications such as analytes precipitation. Therefore, highly sensitive sensors are recommended; in this case, EN and IDE are preferable to ISE.

Table 1 reports  $\text{NH}_4^+$  content in soil extracts measured with different sensors. Ammonium concentration measured by ISE in soil extracts, previously alkalized and measured with EN, showed that some  $\text{NH}_4^+$  was still present in solution after alkalization, as it was presumed by the mild alkaline pH (9.5) obtained after MgO addition. Then the conversion of  $\text{NH}_4^+$  to  $\text{NH}_3$  was not exhaustive. The addition instead of NaOH would result in about pH 12 solution, which would induce  $10^6$  times higher  $\text{NH}_3$  concentration, i.e. almost all  $\text{NH}_4^+$  would be converted to  $\text{NH}_3$ , that is a desirable condition. Nevertheless, the use of NaOH, instead of MgO, is not recommended in soil extracts for its effect on hydrolysis of N-containing organic compounds for soil extracts.

**TABLE 1.** Ammonium concentrations ( $\mu\text{g g}^{-1}$  of dry soil) in the various soil samples measured with different sensors and diverse methods. Data are reported  $\pm$  their relative coefficient of variation.

Samples	ISE		IDE	EN
	/	$\text{MgO}_{\text{EN}}$	MgO	MgO
Soil	268 $\pm$ 3	9.6 $\pm$ 0.5	102 $\pm$ 13	142 $\pm$ 3
Soil +250 mM $\text{NH}_4^+$	622 $\pm$ 1	59 $\pm$ 11	135 $\pm$ 10	1026 $\pm$ 11
Soil +500 mM $\text{NH}_4^+$	547 $\pm$ 1	28 $\pm$ 16.1	118 $\pm$ 8	2232 $\pm$ 18
Soil +2 M $\text{NH}_4^+$	557 $\pm$ 1	0.8 $\pm$ 0.3	141 $\pm$ 15	9476 $\pm$ 22

On the base of data reported in Table 1, EN seemed to be the most reliable sensor. It showed an increase in  $\text{NH}_4^+$  ( $\text{NH}_3$ ) concentration detectable in soil which was significantly correlated with the amount of  $\text{NH}_4^+$  added to soil ( $r^2 = 0.999$ ,  $P < 0.001$ ). The results obtained with ISE, i.e. the higher  $\text{NH}_4^+$  content in extracts of soil with a lower  $\text{NH}_4^+$  saturation (250 mM) than in

those with a greater saturation, can be probably due to the interference of  $\text{K}^+$  present at high concentration in soil extracts (2 M). This interference is higher when  $\text{NH}_4^+$  is lower (greater aspecific signal) and decreases at increasing  $\text{NH}_4^+$  concentration (greater specific signal). Regardless the absolute value in these treated soils, this sensor measured 621.8  $\mu\text{g}$  of  $\text{NH}_4^+$   $\text{g}^{-1}$  of dry soil, which was only 2.3 times that measured in native soils without  $\text{NH}_4^+$  addition, compared to 7.2 times increment measured by EN. Furthermore, the amount of  $\text{NH}_4^+$  measured in untreated soil was not only greater than that detected by EN, but also than values commonly reported in most of the published studies, where they were instead comparable to measurements by EN. Differently, although IDE showed the highest sensitivity to  $\text{NH}_4^+$  ( $\text{NH}_3$ ), by perceiving up to 50 nM  $\text{NH}_4^+$  in soil extracts, it also showed fast saturation at very low  $\text{NH}_4^+$  concentrations. This fact determined that although soil extracts were diluted 5000 times they were still over the calibration curve, then generating similar values in  $\text{NH}_4^+$  content.

## CONCLUSIONS

Electronic nose, although it was neither specific nor the most sensitive sensing detector, it seemed to be the most reliable perceptive system. This feature may depend on the presence of an array of sensors with different sensitivities. Therefore, as well as the analytes of interest will interact differently with various sensors, also the interferents will bind differently to them. The processing by PCA of these presumably (statistically) different fingerprintings of signals, especially if compared with those of standards in calibration curves, will allow to discard the contribution due to background noise or aspecific interferences, thus obtaining information more strictly dependent on the analyte measured. Therefore, EN can be reliably used for measuring  $\text{NH}_4^+$  content in soil.

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