

Nitrate framework directive and cross compliance: two case studies from the MO.NA.CO. monitoring network

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Abstract

The Act A4 refers to Articles 4 and 5 of Directive 91/676 / EEC concerning the protection of waters against pollution caused by nitrates from agricultural sources. The A4 Act applies to farms that have land within a Nitrate Vulnerable Zone (NVZ); it also applies to farms, with or without herds, that participate in measure 214 of the Rural Development Plans. The monitoring was performed in two experimental farms belonging to CREA-FLC. Both monitoring sites are located in vulnerable areas designated by the Lombardy Region. In the monitoring period MO.NA.CO., the regional standard of reference were the Decrees of the Regional Government DGR5868 / 2007 and DGR2208 / 2011. The average cost attributable to administrative requirements was about € 600 / year / company and was mainly due to a professional agronomist who prepared the Agronomic Utilization Plan (PUA), assisted the farm in preparing its communication, provided advice and

informed farm managers on regulatory updates. An informal, not systematic survey made possible to detect that the cost to fulfil the obligations of communication can be very variable depending on the farm's characteristics (size, production, size of the herd) and the type of consultant assisting the breeder (freelancer, association, cooperative).

For example, in some cases the cost is based on the area of the farm, in others on the number of animals in the herd; and in other situations, the associations and freelance agronomist apply a flat rate for the compilation of the PUAs which is unaffected by farm characteristics and ranging from a minimum of € 100 / year / company practiced by some farmers' association to a maximum of € 800 / year / company required by some freelance agronomist.

At Baroncina farm the storage capacity of the slurry, during the monitoring period, was not compliant with the law because the volumes produced could not be stocked for 120 days as required by the standard. New storage facilities have been designed and built; the overall cost of implementation of new tanks was about € 50 / m³.

Introduction

The Act A4 refers to Articles 4 and 5 of Directive 91/676 / EEC concerning the protection of waters against pollution caused by nitrates from agricultural sources. The A4 Act applies to farms that have land within a Nitrate Vulnerable Zone (NVZ); it also applies to farms with or without herds that participate in measure 214 of the Rural Development Plans.

Despite the pollution from agricultural activity is decreasing in recent years (OECD, 2006, 2012), diffuse pollution of ground and surface water for excess of nitrogen and phosphorus is still one of the most serious environmental problems of modern intensive agriculture, contributing to eutrophication of both aquatic and terrestrial natural ecosystems and reducing the quality of drinking water in many areas.

The problem is worsened by the strong concentration of agricultural and livestock activities occurred over the last decades in many developed countries. The concentration process leads to a risk of excessive average production of manure per animal per hectare and increases their content of N and P.

The objective of Directive 91/676/EEC (Nitrates Directive) is to protect water against pollution by nitrates from agricultural sources also improving the exploitation of the nutrient content of manure.

Articles 4 and 5 of the of Directive 91/676/EEC (Nitrates Directive) bind the member states to designate, based on the criteria set by the directive, the NVZs and to establish codes of good practice, train and inform farmers, adopt action programmes.

Should be considered NVZs the areas of land which drain into polluted waters or waters at risk of pollution and which contribute to nitrate pollution.

The Nitrates Directive sets limits on the basis of which Member States have to identify polluted waters, as follows: surface freshwater, in particular that one used or intended for the abstraction of drinking water, containing or that could contain (if no action is taken to reverse the trend) a concentration of more than 50 mg/L of nitrates; groundwater containing or that could contain (if no action is taken to reverse the trend) more than 50 mg/L of nitrates; freshwater bodies, estuaries, coastal waters and marine waters, found to be eutrophic or that could become eutrophic (if no action is taken to reverse the trend).

In Italy, the commitments imposed on farms are reported in the regional programs of action, or, in the absence of such instruments, reference is made to MIPAAF Decree of April 7, 2006, entitled 'Criteria and general technical standards to discipline regional use referred to in Article 38 of Legislative Decree 11th May 1999, No. 152.'

The farmers have to comply with four types of commitments:

- A. administrative commitments (communications);
- B. obligations arising from the storage of manure;
- C. obligations related to the maximum N from manure provided;
- D. limitation concerning the use of effluent (in space and time).

Administrative commitments

For farms having more than 50% of land in NVZ, the administrative requirements are:

To communicate the Operative Plan (POA) which is valid for not more than five years;

To keep a copy of the printed communication to the local competent administration within the farm;

Yearly update of the Agronomic Utilization Plan (PUA);

To integrate / update the documentation in the event of substantial changes;

To prepare and update the monitoring plan.

The PUA should contain elements suitable for the formulation of a nitrogen balance of the soil-plant system:

- i) foreseeable nitrogen requirements of the crops;

- ii) nitrogen supply to the crops from the soil and from fertilization.

Nitrogen requirements of crops are calculated, approximately, through the use of balance method, by the use of an equation reported by the Decree. For the determination of the manure nitrogen available for crops, the Decree of 7th April 2006 of the Italian Ministry of Agriculture establishes standard values of nitrogen excretion for different species and categories of animals. These standards are defined by fixed coefficients that estimate excretions depending on the consistency of herds, but the decree states that if a farmer considers that the standard values are invalid for his herd, he can use different values; different values must be justified presenting a technical-scientific report setting out in detail:

- materials and methods used for the definition of the nitrogen balance based on the measurement of food consumption, retentions in products and volatilization losses; the balance must be drawn up following the directions contained in scientific reports and manuals listed by regions. Alternatively, analytical values found in the effluent can be used to justify different values of nitrogen excreted by a herd; in this case, methods and the sampling plan adopted must be documented and provide:
- results of studies and research reported in scientific journals to demonstrate good reliability of the used data;
- monitoring plan for checking the maintenance of the declared values over time.

Commitments related to the manure storage

Farmers that produce and / or use livestock manure must:

- have adequate storage structures sized in function of the production capacity of the farm, type of manure produced and constraints set by the winter stop;
- ensure the impermeability of the system and the absence of losses and/or leakage.

Compliance with the maximum of nitrogen allowed on the land

The average amount of nitrogen from livestock manure that can be utilized on the land inside a NVZ is 170 kg/ha per year; nitrogen from manure must not exceed 340 kg / ha / year on land falling in not vulnerable area. On both vulnerable and not vulnerable areas, the maximum amounts of nitrogen should be divided and distributed according to the needs of crops.

Limits in space and time concerning the use of effluents

Farmers that produce and / or use livestock manure must respect spatial and temporal bans on the use of nitrogen fertilizers.

The spatial boundaries for the use of livestock manure result from the proximity of water bodies and special agri-environmental characteristics of the land; time limits are generally imposed by the autumn-winter period because of high rainfall.

As a result of the application of the Nitrates Directive benefits on environment are expected at supra-farm dimension; these effects cannot be monitored at farm level; at farm level we can only, partially, estimate costs for the farmer even though it is not always easy to identify direct and indirect costs specifically attributable to the application of rules. However monitoring the compliance with the Nitrates Directive in the farms has a high degree of complexity involving all operational areas from crop production, through the feeding of animals, the storage of manure, and their use; thus MO.NA.CO monitoring has been limited on detecting the costs attributable to the fulfilment of legal obligations (communication, storage, spreading) and focused on few selected indirect environmental indicators such as nitrogen excreted and nitrogen balance at farm level.

Materials and methods

Location of monitoring sites

The monitoring was performed from January 2012 to July 2014 in two experimental farms belonging to CREA-FLC, namely: Cascina Baroncina in Lodi, and Porcellasco Farm in Cremona. Both farms are located in areas of high and medium vulnerability level, in Lombardy Region.

Cascina Baroncina farm

The farm Cascina Baroncina is situated (45° 19 'N, 9° 03' E) in the Po valley, about 2 km from the city of Lodi and has an area of 45 hectares. The soils are sandy-loam, have good drainage, somewhere low and moderate permeability. The soils, in this area, have sub-acid reaction; they have poor contents of nitrogen, low content of potassium and average content of phosphorus. The area is characterized by temperate climate with cold winter, humid and sub-continental type; It is typical of the 'Po Valley' with average annual rainfall of about 800 mm well distributed throughout the year and annual average daily temperature of 12.5°C (Borrelli and Tomasoni, 2005). During the monitoring period the precipitation was abundant, beyond the usual. The main activity of the Baroncina farm is dairy farming. On average, there are about 130 Italian Friesian cattle: 70 young animals and 60 cows; the farm produces about 600,000 kg of milk a year on average. In 2013, the farm was ranked in first place for average production per cow (13,030 kg of milk, 432 kg of protein) in the context of the farms in the provinces of Milan and Lodi.

Porcellasco farm

Porcellasco farm is located about 6 km from the city of Cremona (45° 10 'N 10° 04' E); it covers 82 hectares. The land is flat, of medium texture and very fertile. The main activity is milk production. The main crops are alfalfa for hay and corn for silage; on average, 80 cows and 75 young animals are kept, each year; cows produce, on average, about 630,000 kg of milk per year; the average number of cows in production is close to the national average but below the regional and provincial averages for Italian Friesian herd. The average milk production in 2013 amounted to 8725 kg / cow.

Monitoring scheme

Commitments

Costs for equipment, materials and services attributable to the fulfillment of commitments related to the Act A4 were recorded.

Nitrogen farm balance

The annual balance between needs / removal of nitrogen and nitrogen / brought to the ground gives the value of kg / ha / year of nitrogen surplus.

The budget of nitrogen was estimated using the equation suggested by the MiPAAF Decree of April 7, 2006:

$$N_c + N_f + A_n + (K_c * F_c) + (K_o * F_o) = \sum (Y * B)$$

Excreted nitrogen

The calculation of nitrogen excreted was monitored for lactating cows because this is the category that produces the largest share of N from manure, and because it is one for which it is common to find, at

the farm level, data that serve as indicators of impact and efficiency (production and milk composition).

The calculations for estimating the nitrogen excreted in the monitoring period were based on the method proposed by ERM (ERM/AB-DLO, 1999).

N excreted daily was calculated as balance between nitrogen ingested, nitrogen retained as body nitrogen and nitrogen in the milk product; nitrogen ingested daily was estimated in function of milk production and live body weights (LBW) using equations from literature and knowing the dry matter and crude protein of diets fed; LBW was measured on a sample of animals in one of the two farms; crude protein of total mixed rations was analytically determined on representative samples taken every three months.

Nitrogen produced or lost as a change in body weight was estimated using data about annual variation of the consistency, the percentage content of protein change of weight, was derived from literature.

Nitrogen in milk was calculated according to the amount of milk produced daily and its composition as estimated by functional tests performed monthly by the breeder association of membership of the two farms.

A reduction coefficient was applied to take into account the amount of nitrogen lost through volatilization during the stay in the barn, removal, storage and treatment of wastewater; the standard value of 28% suggested by Ministerial Decree DM 07/04/2006 was considered because direct measure or convincing literature data were not available.

Equations for estimating excreted nitrogen

The average nitrogen excreted daily from lactating dairy cows was estimated also using empirical equations, as a function of:

- milk produced (MilkYield)

$$N_{\text{excreted}} = 2,82 \cdot \text{MilkYield} + 346 \text{ (Nennich et al., 2005);}$$

- milk urea nitrogen (MUN)

$$N_{\text{excreted}} = (15,46 \cdot \text{MUN} + 193,4) / 1000 \text{ (Zhai et al., 2005).}$$

where MUN (mg dL⁻¹) is the N in the form of urea in milk.

The MUN is related to the content of N urea in the blood and urine. When the ammonia that is produced in the stomachs is not converted into microbial protein from the rumen flora it is absorbed into the bloodstream through the walls of the rumen; the liver converts ammonia to urea which is released in the blood, partly excreted in the urine, milk or uterine fluids, partly recycled in the rumen as saliva. Too low or high levels of blood urea N indicate nutritional problems, which is why this parameter and the N urea in milk, closely related to it, are commonly used as indicators of the appropriate feeding. The delay with which the profile of the level of urea nitrogen in the milk follows that in the blood is approximately two hours; thus the concentration of N in milk is indicative of urea nitrogen in the blood product in the interval between one milking and the other. The determination of N urea in milk, is easily accomplished on bulk milk and on individual milk; it is increasingly associated with the functional control of the dairy cows.

Manure sampling

In the first year of monitoring, a sample from the storage tank of the manure of Baroncina farm was taken; sampling was done on the surface and in depth of the tank; at the end of the monitoring five samples were taken from the valve drain of the tank. The following analyses were made on manure samples: dry matter; total N; P; K; ammonium N.

Results

Commitments for Baroncina and Porcellasco farms

Both monitoring sites are located in vulnerable areas designated by the Lombardy Region. In the monitoring period MO.NA.CO., the regional standard of reference were the Decrees of the Regional Government DGR5868 / 2007 and DGR2208 / 2011.

Administrative commitments

The average cost attributable to administrative requirements was about € 600 / year / company and was mainly due to a professional agronomist who prepared the PUA, assisted the farm in preparing its communication, provided advice and informed farm managers on regulatory updates.

An informal, not systematic survey made possible to detect that the cost to fulfil the obligations of communication can be very variable depending on the farm's characteristics (size, production, size of the herd) and the type of consultant assisting the breeder (freelancer, association, cooperative).

For example, in some cases the cost is based on the area of the farm, in others on the number of animals in the herd; and in other situations, the associations and freelance agronomist apply a flat rate for the compilation of the PUAs which is unaffected by farm characteristics; in these cases, it is ranging from a minimum of € 100 / year / company practiced by some farmers' association to a maximum of € 800 / year / company required by some freelance agronomist. There is also the case of small cooperatives offering the service to members, calculating a cost of 0.05 euro per kg of milk produced per year and holding the cost of milk on the invoices to be paid.

Manure storage

In both farms, the storage capacity of solid manure is higher than the minimum legal requirements (90 days).

As regards the slurry (liquid manure) which is the largest share of the effluents, the storage capacity of the Porcellasco farm was found more than adequate to the needs of a correct agronomic use.

At Baroncina farm the storage capacity of the slurry, during the monitoring period, was not compliant with the law because the volumes produced could not be stocked for 120 days as required by the standard. The existing storage capacity of 581 m³ was enough to contain the waste produced on average on 106 days; to meet the requirements of the law and to avoid risks of overflowing in the event of particularly abundant rainfall it was necessary to achieve a storage capacity equal to 657.80 m³. The abundant winter rains occurred during monitoring, contributed substantially to fill the old tank containing the slurry. For these reasons, new storage facilities have been designed and built; a new storage tank with a capacity of 1000 m³ in anticipation of impending changes in the management of the company that will require a capacity larger than needed at present; building of new tank was finished in the year 2015.

The overall cost of implementation in the new tanks was about € 50 / m³. The cost for the realization of 657.80 m³ of storage required by the standard can then be estimated at € 32,890, but the mere cost of the adjustment in terms of storage capacity should be estimated at € 3,840.

Compliance with the maximum of nitrogen allowed on the land

Baroncina farm

The acreage of Baroncina farm insists for the most part (34.04.57 ha) on a NVZ; the load of cattle and milk production are considerable and should lead to an excess of excreted nitrogen; since the company

(CREA) owns neighbouring farms insisting on nonvulnerable areas, the excess of manure nitrogen was distributed on these lands; this allowed to reach an annual maximum amount of distributable nitrogen from manure of 14634 kg; since the amount to be distributed was estimated to be 9117 kg (6247 kg of slurry) the farm complies with rules.

Moreover, 10,840 kg of N of mineral fertilizers could be used.

Porcellasco Farm

Table 1 shows the maximum amount of nitrogen derived from farm's effluents that was possible to distribute on fields of Porcellasco farm.

Since the total amount of N excreted calculated on the basis of herd composition and standard coefficients was 9,470.40 kg/year (Table 2), the farm was complying with the standard; moreover, there was the possibility of distributing 12754.51 kg nitrogen from mineral fertilizer.

Limits in space and time concerning the use of effluents

In the period of monitoring, the temporal limits, associated with the scarce storage capacity and exceptional rains brought the management system of the effluents in times of high criticality that were overcome by increasing the capacity of storage.

Farm nitrogen balance

At Baroncina farm the total value of needs of crops and related nitrogen inputs is 8404, 2 kg / year; the removals are estimated at 10,603 kg / year, so the budget was negative as had to be expected, since the fertilization plan is designed in accordance with the standard; the crop needs were calculated as in Table 3.

Also at Porcellasco farm the nitrogen balance between needs of crops and contributes from animal and chemical sources was slightly negative because chemical fertilisations were made in a way that needs were not exceeded.

Excreted nitrogen

Live weight

The average live weight of 65 dairy cows (33 primiparous and 28 multiparous) weighted at Porcellasco farm was 630,25 kg (s.d. = 68.81).

Baroncina Farm

The sample analysis of the ration showed a high protein content (16.2 to 17.5% dry matter); there is room for a reduction in the N content in the rations and an improvement of the N utilization efficiency.

The trend of the estimated values of nitrogen to the field from lactating cows and parameters used for the estimate in the monitoring period are shown in Figure 1.

The average value of nitrogen to the field and its standard deviation were found to be 88.00 and 4.74 kg / head / year. The average excretion calculated is a value very close to the average value shown in Table C1 of Annex 3 of DGR 5868 (http://www.ersaf.lombardia.it/upload/ersaf/gestionedocumentale/dgr%208_5868_2007_784_4457.pdf); however, in comparison with the table C1, a higher level of efficiency of nitrogen transformation was achieved; in fact a comparable level of excretion was obtained with higher ingestion of N (greater content of protein in the diet and increased level of ingestion) balanced, however, by a higher production of milk with a high content of protein; the efficiency of nitrogen utilization was 0.33, higher than that derived from Table C1, ranging between 0.26 (1st quartile) to 0.28 (4th quartile) with a mean value of 0.27.

The mean value obtained from the equation that estimates the nitrogen excreted as a function of the milk production value is 97.3 (SD = 2.0) kg / head / year; this value would place Baroncina Farm in the fourth quartile of the population represented in Table C1 and highlights that the production only, until you consider how efficiency has been obtained, it is not a good indicator of the emission of nitrogen.

The equation that estimates nitrogen excreted as a function of MUN produces an average figure of 78.0 (SD = 4.8) kg / head / year; compared to the previous, the equation provides, therefore, values closer to those obtained with the method of the budget and is able to calculate, at least in part, the variability due to different efficiency of utilization of food protein; on the other hand it does not take into account the absolute levels of N ingested and production, thus may underestimate, in some conditions, nitrogenous emissions.

The range of MUN expected in cows fed with the recommendations of NRC is 10-16 mg / dL, the mean value found in the monitoring was 11.32 mg / dL.

Porcellasco Farm

The average values of net nitrogen excreted amounted to 73.17 (SD = 7.01) kg / head / year with an average efficiency of 0.30.

Composition of cattle slurry

The average chemical composition of the slurry sampled from the storage tank of Baroncina farm is shown in Table 4.

Discussion and perspectives

The monitoring showed that the major economic and technical constraints were due to spatial and time limitation in using effluents; in one of the two farms, they have made necessary to expand the storage tanks, while the other farm did not incur in such costs in the period of monitoring only because the storage facilities were been adequated at an earlier time.

In addition, the monitoring showed that complying with law does not necessarily leads to good behaviour in terms of environmental impact; for example, the amount of nitrogen excreted is generally calculated through the use of tabulated values depending on the species and housing. These values correspond to those most frequently encountered as a result of direct measurements carried out in many herds belonging to a wide range of cases; these averages have been useful for the application of the rules especially in a first implementation phase but they are not rewarding the most efficient farmers.

European, national and regional rules provide that individual farm can prove excretion other than tabulated; the interest of farmers in using customized data rather than tabular ones is linked to their ability in implementing good techniques and practices to reduce emissions of nutrients.

For example, the relationship between intake and nitrogen excretion in dairy cows is well documented and shows that:

Increasing productivity, increases the efficiency of nitrogen utilization due to dilution of the maintenance share; the increase in production increases the ingestion and urinary excretion but also the efficiency of utilization. The conversion efficiency in milk can vary between 21 and 43%, which means that from 57% to 79% of the nitrogen ingested may be found in excreta. In ruminants, the efficiency of utilization of nitrogen from feeds is a function of the efficiencies of utilization of nitrogen in the rumen and of utilization of amino acids absorbed in the intestine.

Digestion influences the content of N in the faeces, metabolism affects the urinary nitrogen.

The composition of diets (the type and quantity of feeds fed daily) and its physical form affect both digestion, and metabolism of N.

The excess of N excreted in dairy cattle can result:

Table 1. Maximum values of nitrogen applicable to Porcellasco farm.

Category	Acreage Area (ha)	Max total N (kg)	Max standard efficient N
Total non-vulnerable area	7.99.68	2718. 91	17374.72
Total vulnerable area	68.46.72	11,639. 42	
Total	76.46.40	14,358.33	

Table 2. Total effluents available at Porcellasco farm.

Liquid manure (slurry)		Solid manure					
m ³	N (kg)	P ₂ O ₅ (kg)	K ₂ O	m ³	N (kg)	P ₂ O ₅ (kg)	K ₂ O, kg
2905,62	8320,46	4722,81	7593,05	476,55	1149,94	900,41	1446,81

Tabella 3. Calcolo dei fabbisogni culturali per l'azienda Baroncina

	ha	Nc (kgN/ha/ year)	Nf (kgN/ha/ year)	OM (%)	Ans (kgN/ha/ year)	Ana (kgN/ha/ year)	Organic fertilizations (kgN/year)	Chemical fertilizations (kg urea)	Fc (kg/ha/ year)	Kc	Fo (kgN/ha/ year)	Ko	Needs (kgN/ha/ year)	Needs (kgN/year)
Alfalfa	9.31	80	32.92	1.60	0	30							142.9	1330.3
Temporary grasslands	8.95	40	32.92	1.60	0	30	2273.8				254.1	0.45	217.2	1944.3
Permanent meadows	6.09	60	32.92	1.60	0	30	1546.2				254.1	0.45	237.2	1443.9
Ryegrass	6.44	0			0								0.0	
Corn	13.60	0	32.92	1.60	48	30	3456.1	600	44.1	0.46	254.1	0.55	270.9	3685.8
Total							7276.1						868.3	8404.2

Nc, soil's nitrogen availability from previous crops; Nf, soil's nitrogen availability from organic fertilizations; OM, organic matter; Ans, natural sources of nitrogen; Ana, nitrogen from atmospheric sources; Fc, nitrogen from chemical fertilization; Kc, efficiency coefficient of Fc supply; Fo, nitrogen from organic fertilization; Ko, efficiency coefficient of Fo supply.

- By the contribution of degradable N in excess of requirements of rumen microorganisms;
- From amino acid imbalance in the coverage of the needs of the animals;

In both cases the excess of N is catabolized to urea that diffuses into the body and is excreted in the urine. Urea constitutes 10 to 80% in urinary nitrogen. All feeds and nutritional strategies that promote the efficiency of the use of nitrogen in the rumen and post-rumen are helpful in reducing the amount of nitrogen in excreta, and first of all it is useful to compensate in an accurate and non-redundant way the needs of the animals.

The loss of nitrogen resulting from ruminal degradation of dietary protein can be reduced by optimizing the diet and favouring the production of microbial proteins which are effectively used in the intestine. The production of rumen microbial proteins must be helped by an optimal ratio of degradable and non-degradable protein in the rumen in accordance with the needs of micro-organisms for protein synthesis and an adequate supply of energy in the rumen.

Also analysis of the farm effluents could be used to customize data; the analysis was not widely used as a management tool because there

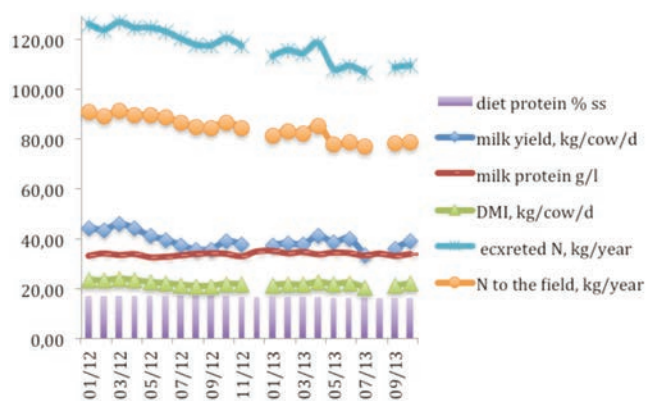


Figure 1. Nitrogen balance and nitrogen to the field.

Table 4. Chemical composition of manure sampled at Baroncina farm.

Parameters	Average	C.V. (%)*
Dry matter (S.S.) (g / 100g)	3.53	41.28
Nitrogen (g / kg tq)	32.11	1.64
Nitrogen (g / kg DM)	47.71	27.82
Phosphorus (mg / kg t.q.)	20.66	1.49
Phosphorus (mg / kg SS)	5692	816.41
Potassium (mg / kg t.q.)	1.472	428.38
Potassium mg / kg SS	43346.12	11.88
Ammonia nitrogen (g / kg t.q.)	0.86	26.48
Ammonia nitrogen (g / kg S.S.)	25.452	13.39

The average and CV resulted from analysis of five samples taken from 16 June to 23 July 2014.

was a widespread belief that they produce inaccurate results due to the difficulty of estimating the volumes stored and to obtain representative samples. The scarce diffusion of the analysis of the effluents at farm level was also due to the high laboratory costs.

New prospects are now opening with the refinement of quicker and cheaper methods to analyse effluents; NIR spectroscopy is particularly promising (Cabassi *et al.*, 2015; Finzi *et al.*, 2015). The content in dry matter, organic carbon Kjeldahl nitrogen, ammonia nitrogen can be estimated directly as a function of their absorption in the near infrared region; other constituents, such as P and ashes that have poor absorption in this region may also be well estimated because highly correlated to the other nutrients (Cabassi *et al.*, 2015).

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