FATE OF PATHOGENS IN SOILS AND PLANTS IN A LONG TERM FIELD STUDY AMENDED WITH DIFFERENT COMPOSTS AND MANURE

Brochier V.[1], Kallassy M.[2], Poitrenaud M.[1], Houot S.[3]

[1] Veolia Environnement R&D, 291 av Dreyfous Ducas, F-78520 Limay, France.
Tel : +33(0)130982954 violaine.brochier@veolia.com, maelenn.poitrenaud@veolia.com

[2]Veolia Environnement Services, Nanterre, France. monique.kallassy@veolia-proprete.fr

[3]INRA, UMR Environment and Arable Crops, F-78850 Thiverval-Grignon, France.
Tel: +33(0)130815401 sabine.houot@grignon.inra.fr

1 INTRODUCTION

The innocuousness and the agronomic efficiency of organic amendments must be demonstrated prior to their application on cultivated field, in order to protect soils and plants qualities. For example, pathogens that can be present in raw materials before composting represent a potential risk of contamination of crops and consequently a potential risk for consumers. Most countries lack of legal regulations defining the hygienic requirements for finished composts. In most cases, indirect parameters, e.g. maturity of the product, frequency of turning of windrows, temperature-time relationships are kept voluntary or in the framework of quality networks (EC, 2002; Böhm, R. 2007). In France, the standards NFU 44-095 concerning compost containing sewage sludge and NFU 44-051 concerning the other organic amendments impose thresholds for metallic and organic pollutants and also for micro-organisms contents in the finished compost (AFNOR 2002 and 2006). Pathogens (*Salmonella* spp, *Listeria monocytogenes*, Helminths eggs) and indicators of treatment efficiency (*Escherichia coli*, *Clostridium perfringens*, *Enterococcus*) are distinguished (Table 1). The analytical methods used in French standards correspond to those used for food.

The composting effect on pathogens survival was studied in previous works (Lemunier et al., 2005). Survival of pathogens from amendments in soil or their potential transfer to plants has been described but mainly concerned non composted organic amendments (Pourcher et al., 2006). In contrast, few works concern their potential transfer from composts to soils and plants (Islam et al., 2004).

The objective of our study was to determine the potential contribution of three urban composts (municipal solid waste, biowaste and green waste composted with sewage sludge) and farmyard manure both to the short and midterm impact on pathogens concentrations in soil and their potential transfer to plants.

TABLE 1 **Maximum levels of pathogenic germs in the French compost standards (concentrations for wet weight)**

French standard	NFU 44 095 Sewage sludge compost		NFU 44 051 Other composts and farmyard manure	
	Max. level	*utilization*	*Max. level*	*Utilization*
E.coli	10^3 per g	Marketing gardening	10^2 per g (only advised)	All crops
	10^4 per g	Arable crops		
C. perfringens	10^2 per g	Marketing gardening		
	10^3 per g	Arable crops		
Enterococcus	10^5 per g	All crops	10^4 per g (only advised)	All crops
Salmonella	0 in 25 g	Marketing gardening	0 in 25 g	All crops
	0 in 1 g	Arable crops	0 in 1 g	All crops
L. monocytogenes	0 in 25 g	Marketing gardening		
	0 in 1g	Arable crops		

2 MATERIALS AND METHODS

2.1 Field experiment

The "QualiAgro" long term field experiment has been initiated in 1998 in order to study the agronomic and environmental effects of repeated urban compost applications on soil and plant qualities. The field is located at Feucherolles, Ile de France, 35 km west of Paris (Houot et al., 2002). The field experiment is divided into 4 replicates of 10 plots of 450 m² where three composts (municipal solid waste compost: MSW; sludge co-composted with greenwastes: GWS; biowaste compost: BIO) are applied and compared to a farmyard manure (FYM) and to control non amended treatment (CTR) with or without additional mineral N fertilizer. Composts and manure had been applied every two years since 1998 at doses equivalent to 4 t of C/ha, corresponding to 15 to 20 t Dry Weight (DW) /ha depending on the organic products. The field experiment is cultivated with a wheat-maize succession. In 2006-2007, because of a *Diabrotica virgifera* alert, barley was seeded in replacement of maize and organic amendments were applied after harvest in September 2007.

2.2 Organic amendments, soils and plants sampling

Composts and farmyard manure were sampled the day when they were applied. They differed by their stability level and their chemical characteristics (Houot et al., 2002). Their organic matters varied between 34 to 57 % DW in order: BIO < GWS < FYM, MSW. This wais consistent with the duration of composting: respectively 6, 4.5 and 1 month(s) for BIO, GWS and MSW and no composting for FYM. Representative samples of soils were collected from pooled samples in each plots of the mineral N complemented part of the field (20 plots) before amendment application, at each harvest and at different times after application (Table 2). Plants were sampled every year at the harvest in the fertilised part of the experiment (20 plots). Each plant was immediately split into three fractions: roots, stems and leaves, and grains. For maize, grains were isolated from cobs. For wheat and barley grains were not separated from the ears. The samples were kept at 4°C before analysis. Samples were crushed and analysed following the standard methods.

TABLE 2 **Summary of all sampling dates**

	2004			2005		2006			2007		2008	2009
	Sep	Oct	Nov	Mar	Sep	Jul	Sep	Nov	Jul	Sep	Oct	Jul
Time after spreading *months*	0	1	2	6	12	22	0	2	10	0	13	22
Organic amendments	X[1]						X[1]			X[1]		
Soils	X[1]	X[1]	X[1]	X[1]	X[1]	X[1]	X[2]	X[2]	X[1]		X[2]	X[2]
Plants					maize[1,3]	wheat[1,4]				barley[1,4]	Maize[2,3]	Wheat[2,4]

[1] All the micro-organisms; [2] *Enteroccocus* and helminths eggs; [3] Roots, stems+leaves, grains; [4] Stems+leaves and grains.

2.3 Microbial analysis of organic amendments, soils and plants

The microbial analysis of composts, soil and plants was done following the standard methods given in the French quality standards (NFU 44-095 and NFU 44-051) (Table 1). *E. co*li were counted on Tryptone bile glucuronide medium (TBX) (NF V 08-053). *C. perfringens* level (vegetative forms plus a great part of spores) was estimated according to method NF V 08-056 after sowing on tryptose sulfite medium. *Enterococcus* were estimated from the micro-organism density after sowing and incubation of microplates and examination under UV radiation (NF IN ISO 7899-1). *L. monocytogenes* were counted in 25g after enrichment by Faser bubble, incubation and identification on PALCAM gelose medium (NF V 08-055). According to NF V 08-052, search for *Salmonella* was performed in 25g, by biochemical identification after various steps of enrichment and incubation. Total helminths eggs analysis were performed in 1.5g according to a densimetric method (XPX 33-017), by triple floatation in a zinc sulfate solution.

3 RESULTS AND DISCUSSION

3.1 Composts and manure

No *Salmonella* nor *L. monocytogenes* were detected in the composts and manure, in 2004, 2006 and 2007. Helminths eggs were not detected in the amendments, except in FYM in 2004. *E. coli* was not detected ($<10^2$ CFU/g), except in GWS and FYM in 2006 (10^2/g). *C. perfringens* levels varied between $1.6.10^1$ (GWS) and $6.4.10^3$ (FYM) CFU/g (Table 3). *Enterococcus* levels varied between $9.1.10^3$ (BIO) and $1.0.10^6$ (MSW) MPN/g.

The three composts matched the microbial values recommended by the French standards NFU 44-051 or NFU 44-095. Higher concentrations of micro-organisms were detected in MSW and in FYM. This may be explained, respectively by the short time (1 month for MSW) or the absence of composting (FYM) of these two amendments. FYM matched the microbial values recommended by the French standards NFU 44-051, except in 2004 (helminths eggs detected).

TABLE 3 **Concentrations of micro-organisms in composts and farmyard manure: means (standard deviations) or absence/presence (a / P) (2004, 2006 and 2007)**

	E.Coli CFU/g		*C. perfringens* CFU/ g		*Enteroccocus* (MPN)/g		*L. monocy-togenes*/25g	*Salmonella* spp /25g	Helminths eggs /1,5g
MSW	$<10^2$	(0)	$1.6 .10^2$	($1.3 .10^2$)	$1.0 .10^6$	($1.6 .10^6$)	a (3/3)	a (3/3)	a (3/3)
BIO	$<10^2$	(0)	$2.1 .10^2$	($7.1 .10^1$)	$9.1 .10^3$	($8.8\ 10^3$)	a (3/3)	a (3/3)	a (3/3)
GWS	$<10^2$	(5.10^1)	$1.6 .10^1$	($1.0 .10^1$)	$1.7 .10^4$	($2.0 .10^4$)	a (3/3)	a (3/3)	a (3/3)
FYM	$<10^2$	(5.10^1)	$6.4 .10^3$	($7.6 .10^3$)	$2.1 .10^5$	($1.5 .10^5$)	a (3/3)	a (3/3)	P (1/3)

3.2 Soils

L. monocytogenes and *Salmonella* were never detected in soil samples, except once in 2006, where *Salmonella* was detected in one of the 3 plots BIO (1/3). *E. coli* levels were under the detection limits (10^2 CFU/g) in all soil samples and all treatments. The presence of helminths eggs occurred only rarely in soils and showed no obvious correlation with soil treatments. Indeed, they were detected in CTR (1/3) and BIO (1/3) in October 2004 (1 month after spreading); in FYM (1/3) and MSW (1/3) in November 2004 (2 months after spreading); in FYM (3/3), MSW (2/3) and GWS (1/3) in March 2005 (6 months after spreading); GWS (1/4) in July 2007 (10 months after spreading). In contrast, no helminths eggs were detected in soils in 2006, 2008, 2009. *C. perfringens* was detected in all plots, without significant difference between treatments. Their levels varied between : $<1.10^1$ and $2.9.10^2$ (Figure 1). The significant decrease observed in winter 2005 could be explained by the rather cold winter period (dayly average temperatures lower than 10°C during more than 2 months) before the sampling date (March 2005). Probably *C. perfringens* may have survived under spore form when restrictive climatic conditions occurred. The temperature increase during summer allowed probably spore germination and growth of vegetative forms explaining the concentration increase up to 10^2 CFU/g at September 2005, July 2006 and July 2007, which were close from the content before spreading (de Jong, 2003). *Enteroccocus* levels ranged from $1.1.10^2$ up-to $1.7.10^3$ MNP/g in all soils without significant difference between treatments and without temporal variation.

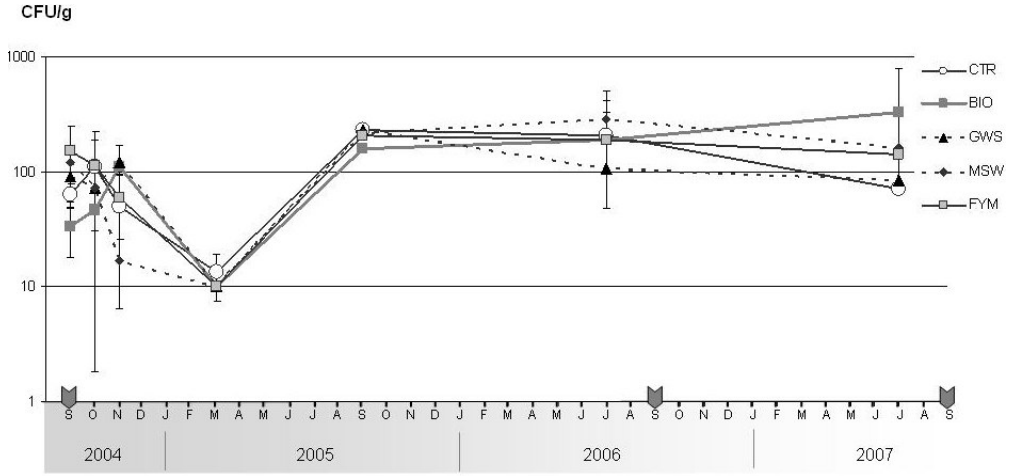

FIGURE 1 **Concentrations of *C. perfringens* in soils** (3 or 4 replicates at each date, logarithmic scale, differences lower than 1 log are considered as no different, arrows mean amendments applications)

3.3 Plants

Although some cautions were previously pointed out concerning the reliability of analytical methods applications to composts, these methods coming from food standards are consequently adapted to plants analyse.

No trace of *Salmonella* was detected whereas *Listeria* and *E. coli* were under the detection limits (10^2 CFU/g) in all the parts of the plants and whatever the treatment. In contrast, *C. perfringens* were under the detection limits (10 CFU/g) in stems, leaves and grains, but they were close from the detection limit in maize roots (between 10 and 15 CFU/g). *Enteroccocus* were detected in all parts of plants, with higher concentrations in stems and leaves ($1.7.10^3$ and $2.3.10^4$ MNP/g) than in roots ($7.1.10^2$ and $2.3.\ 10^3$ MNP/g) or grains ($2.2.10^1$ and $2.4.10^4$ MNP/g). This may be because the method used was not sufficiently efficient enough to discriminate between different *Enterococcus* species. This implied that species which can be found in plants are not necessary human pathogens (Whitman et al. 2005). Helminths eggs were never detected in grains and stems + leaves, except in stems + leaves in 2006 in BIO (1/4), GWS (1/4) and TEM (2/4). They were detected in maize roots for all the treatments and only once in wheat root (BIO). As for *Enterococcus*, this result could be explained by the presence of cysts of other helminths (parasites of plants). Indeed, the method used was not specific to the human and animal parasitic nematodes leading to the recovery of plant parasitic nematode eggs in the root environment that probably offers favourable conditions for cyst hatching.

4 CONCLUSIONS

Some interrogations remain concerning the reliability of results obtained with methods issued from food standards because the compost matrix is very different to alimentary matrix, and an important work must be carried out in order to guarantee the accuracy of methods used for compost analysis. This work is currently carried out within the framework of the European project HORIZONTAL for some parameters.

Even if results regarding the presence of human pathogen in composts have to be cautiously interpreted, they can be discussed. The composts matched the French standards for microbial pathogens content in composts. No significant effect of composts application in field conditions was observed on pathogens levels in soils and plants, especially in grains. Based on current French standards, the use of composts with microbial quality matching the legislation criteria seems to be safe with no risk of soil and plant contamination

ACKNOWLEDGEMENTS

The authors are grateful to G Bodineau, JN Rampon, M Jolly, R Lecarpentier and Y Crepel, for their essential action on the field experiment and to AS Lepeuple, J Doublet for their reading.

REFERENCES

AFNOR 2002. French standard U 44-095, Organic soil improver composts containing substances essential to agriculture, stemming from water treatment, AFNOR Editions, St Denis La Plaine, France.

AFNOR 2006. French standard U 44-051, Organic soil improver composts, AFNOR Editions, St Denis La Plaine, France.

Böhm R 2007. Pathogenic agents. In: Compost Science and Technology. LF Diaz, M De Bertoldi, W Bidlingmaier and E Stentiford. Waste Management series 8 : 177-200.

De Jong AEI 2003. Clostridium perfringens : spores and cells, media and modelling, PhD.Thesis, Wageningen Universiteit [online] http://library.wur.nl/wda/dissertations/dis3462.pdf

European Commission 2002. Regulation (EC) n° 1774/2002 of the European Parliament and of the Council of 3 October 2002, laying down health rules concerning animal by-products not intended for human consumption. Official Journal of the European Communities, L273, 1-95.

Houot S, Clergeot D, Michelin J, Francou C, Bourgeois S, Caria G, Ciesielski H 2002. Agronomic value and environmental impacts of urban composts used in agriculture. In : Insam, H., Riddech, N., Klammer, S. (Eds), Microbiology of Composting. Springer-Verlag Berlin Heidelberg, 457-472.

Islam MJ, Morgan J, Doyle MP, Phatak SC, Millner P, Jiang X 2004. Fate of Salmonella enterica serovar Thyphimurium on carrots and radishes in fields treated with contaminated manure composts or irrigated water. Appl. Environ. Microbiol., 70, 2497-2502.

Lemunier M, Francou C, Rousseaux S, Houot S, Dantigny P, Piveteau P, Guzzo J 2005. Long-term survival of pathogenic and sanitation indicator bacteria in experimental biowaste composts, Appl. Environ. Microbiol., 71, 5779-5786.

Pourcher AM, Picard-Bonnaud F, Ferré V, Gosinska A, Stan V, Moguedet G 2007. Survival of faecal indicators and enteroviruses in soil after land-spreading of municipal sewage sludge. Applied Soil Ecology 35(3), 473-479.

Whitman RL, Byers SE, Shively DA, Fergusson DM, Byappanahalli M 2005. Occurrence and growth characteristics of Escherichia coli and enterococci within the accumulated fluid of the northern pitcher plant (*Sarracenia purpurea L.*) Canadian journal of microbiology 2005, vol. 51, no12, 1027-1037.