

# **Health Risks from Pathogens in Livestock Manures**

**Stuart Williams**

*Institute for Animal Health, Pirbright, Woking, Surrey, GU24 0NF, UK. Email:  
stuart.williams@bbsrc.ac.uk*

Bacteria, viruses and parasites from faecal sources such as slurry or solid manure are of environmental importance as they are stored and handled on farms. Treatment of slurry and solid manures to reduce or eliminate these pathogens is not routinely carried out therefore they pose a threat to other animals and the human population.

The operation of large scale farms housing high numbers of animals in one location creates problems with regard to the possible spread of the various diseases. Some pathogens are transmissible to humans (zoonotic) either directly through excrement or by animal products. Such diseases include parasitic diseases, viral diseases and a variety of bacterial diseases. There is a range of health hazards relating to animal wastes due to the presence of a wide range of bacteria and other organisms. These may not be the cause of contagious illness but a series of measures will reduce the health risks to individuals who come into close contact with livestock wastes. Hazards are also associated with the application of wastes to food crops on arable land. The treatment of animals wastes may have to be considered prior to land application so as to allay fears from the food producers and the general public.

# **Measurement of pathogen transfer in aerosols following land application of manure**

**Phil Hobbs, Dave Davies<sup>1</sup>, Jon Williams and Helen Warren<sup>1</sup>**

*Institute of Grassland and Environmental Research, North Wyke Research Station,  
Okehampton, Devon EX20 2SB phil.hobbs@bbsrc.ac.uk*

*<sup>1</sup>Institute of Grassland and Environmental Research, Plas Gogerddan, Aberystwyth, SY23  
3EB, UK*

The study of the transfer of pathogens through the air that may cause disease from manure spreading is complex. Investigation of pathogen transfer by air has been brought to the fore by the concern of increasing numbers of illnesses caused by microbial infections relating to food. Sampling procedures and methods of detection and collection affect potential conclusions concerning transmission. The recent environmental history of the pathogen is important, for example an organism that has previously been exposed to drying and rewetting will be more likely to survive aerosol travel and often exhibit increasing pathogenic capacity.

The principal aims of this study were to evaluate how far pathogens present in aerosols can travel and transmit disease. This will require establishing the best means of sampling that are representative or realistic with the disease transmission capability of different species. Aerosols containing pathogens can travel significant distances, for example pollen that is deposited at the Earth's poles is about the same aerodynamic dimensions as microbes, so pathogen viability is critical. Desiccation is the most difficult problem for microbial species to remain viable. At take-off as either a dry or wet microbe, changes in water content will inevitably follow. The ability to initiate disease depends upon other factors related to the ability to survive and infect the host. Sampling approaches will be reviewed and some early experimental studies that are relevant to the transfer of pathogens (including marker organisms) as aerosols will be used to highlight monitoring difficulties.

Key words; pathogen, aerosol, disease transmission, desiccation.

**Concerted Action AROMIS**  
**Assessment and reduction of heavy metal input into agro-**  
**ecosystems**

**Henning Eckel, Ursula Roth, Helmut Döhler, Ute Schultheiß**

*Association for Technology and Structures in Agriculture (KTBL),  
Bartningstrasse 49, 64289 Darmstadt, Germany  
email: h.eckel@ktbl.de*

Heavy metals which enter agro-ecosystems through various agricultural and industrial activities can accumulate in soils and may have long-term implications for soil health and function, the quality of agricultural produce and the wider environment.

Against this background, the EU financed the AROMIS Concerted Action, set up by KTBL and 23 research institutions from across Europe, representing EU-15 Member States, accession countries and associated states. The consortium aimed to provide a cross national assessment of heavy metals in European agriculture.

The first step consisted of the compilation of data on heavy metal contents in all media relevant for agricultural like e.g. mineral and organic fertilisers, feedstuffs, feed supplements, other input sources like atmospheric deposition, as well as background concentrations in soils. For the documentation and the handling of the data, a database was constructed which additionally contains a compilation of relevant legal regulations on national and EU level.

In a second step heavy metal balances on farm level were calculated for a selection of production types to have an instrument at hand for evaluating the relevance of the various input and output pathways. In addition to these status quo calculations the tool allows the assessment of the effect input reduction measures would have on a given farming system. For the latter purpose a number of scenario calculations using data from existing farms as well as theoretical model farms were carried out.

In a third part of the project the available options for reducing the heavy metal input were evaluated regarding their potential, readiness for implementation, political enforcement, precision and side effects and other relevant criteria. Furthermore the need for research in the area of heavy metals in agriculture was described.

Comparisons between countries regarding metal inputs into agro-ecosystems proved difficult due to differences in monitoring programmes, sample collection and analysis, and the quality and quantity of data collected. Standardised procedures for sampling and analysis are therefore recommended.

The evaluation of the farm gate balances shows that the input of metals in most cases exceeds the outputs leading to a net surplus in the system. The balance results are dominated by the feeding regime in the case of livestock farming systems and by the choice of fertilisers, i.e. the use of sewage sludge and inorganic P-fertilisers. The assessment of the risks associated with a positive balance result requires an additional site specific approach (field scale balances) and a long term modelling of changes in soil and leachate metal contents.

# Hydrated lime and Velox reduce rapidly enteric micro-organisms of manure

Helvi Heinonen-Tanski<sup>1</sup>, Satu Antola<sup>2</sup> and Kjell Weppling<sup>2</sup>

<sup>1</sup>*Department of Environmental Sciences, University of Kuopio, POB 1627, FIN 70211 Kuopio, Finland, heinotan@uku.fi and* <sup>2</sup>*Nordkalk Oyj Abp, Water Group, FIN 21600 Pargas, Finland*

A rapid and effective method for reducing enteric micro-organisms in solid manure or slurry is often needed. Quicklime and hydrated lime have commonly been used for chemical disinfection. In Finland, recommendations for destroying of salmonella given by the Ministry of Agriculture and Forestry suggest a treatment with 30 kg hydrated lime/ton of slurry or urine and a subsequent one week incubation time. For solid manure 2-3 months composting at high temperature is recommended.

Nordkalk Velox is a lime-based product containing reactive oxygen in the form of calcium peroxide. The efficiency of this product to destroy enteric micro-organisms was studied. Hydrated lime was used as a control. The experiments were carried out with slurry and solid manure of turkey, pig and horse.

Doses of 3 – 30 g/kg (or g/L for slurry) were tested on the number of faecal coliforms, enterococci and somatic coliphages. In addition pH was measured.

With cattle slurry and good stirring a dose of 7.5 g/L of both Velox and hydrated lime destroyed micro-organisms to a level below the detection limit. With solid manure doses of 30 g/kg were necessary to reduce the microbial numbers below the detection limit. The higher doses needed may be explained by difficulties to obtain a homogenous mixture between the solid manure and the disinfectant.

Velox was slightly more effective than hydrated lime. The pH is not the only explanation for good sanitation. According to these results a one week treatment time would not be needed, because the disinfection effect was clear already after one to two days.

# Use of Crude Coffee Grain for Treatment of Petroleum Hydrocarbon-Contaminated Soil

**G. Adriana Roldán-Martín and Refugio Rodríguez-Vázquez**

*Centro de Investigación y Estudios Avanzados del IPN (CINVESTAV) Av. Instituto Politécnico Nacional 2508 Col. San Pedro Zacatenco, 07360 Apartado postal 14-740, 07000 México, D.F. , e-mail: aroldanm@iingen.unam.mx, rrodrig@mail.cinvestav.mx*

In Mexico a number of diverse technological options cradles in biological processes have been tested for the restoration of sites contaminated with hydrocarbons, one of them is to add organic bulking agents to improve the characteristics of soil. The present work was performed to evaluate the crude coffee grain as a bulking agents in biopiles, to increase the biodegradation petroleum of hydrocarbons in the contaminated soil.

The first part of the work consisted in desing the biopile and the second part in the evaluation of the biopile. Sampling were take every week during the first month, every fifteen day during the second month and every month during the third month. During the treatment, humidity, pH, organic matter, phosphorus, nitrogen and Total Petroleum Hydrocarbons (TPH) were determined. TPH's were determined by infrared spectroscopy IR taking like reference method EPA 418.1. As an alternative of hydrocarbon recovery of the soil, It was extracted by agitation and centrifugation taken base in the reported by Schwab, (1999).

The conditions of the tests were humidity 30%, soil/agro industrial waste ratio 98:2 and C:N:P 100:10:1. The process consisted of placing the biopile (23 kg) in rectangular glass container and mixed the contaminated soil with the required nutrients and humidity. The used Nitrogen source was  $(\text{NH}_4)_2\text{SO}_4$  and the phosphorus  $\text{K}_2\text{HPO}_4$

The concentration initial of soil was 58 000 ppm of Total Petroleum Hydrocarbons (TPH).At 7th day a decrease in the concentration of TPH's was observed (8%), on 42th day the TPH's concentration continued decreasing (50%), The highest grade of TPH's removal (65%) occurred at 90 days.

With base in the previous it is confirmed that the coffee grain is efficient agro-industrial waste in the removal of TPH's in contaminated soils with hydrocarbons.

## **Hygienic aspects of biosolids reuse**

**Jan Venglovsky, and Jose Martinez\***

*University of Veterinary Medicine, Komenskeho 73, 040 01 Kosice, Slovak Republic*

*E mail: jan@venglovsky.com*

*Cemagref Livestock and Municipal Wastes Management Research Unit 17, avenue de  
Cucillé 35044 RENNES Cedex FRANCE*

*E mail: jose.martinez@cemagref.fr*

Land application of biosolids provides agricultural benefits and presents a cost-effective method of sludge disposal following wastewater treatment. However, reuse of this product causes health concerns that must be addressed and satisfied before land application is an accepted practice. Health concerns include pathogen transmission to food or agricultural workers, contamination of ground water or surface water with faecal material from field runoff, and build-up of heavy metals or organic contaminants.

Survival and transport of pathogens from manure to the environment depend on a number of complex phenomena.

In recent years the fate of human and veterinary therapeutic agents as a potential pollutant of the environment has been paid increased attention. Substantial quantities of these compounds and their metabolites are excreted, flushed down the drain, discarded as waste, or left over in animal feedlots. When they enter the sewer, several of these compounds are not adequately eliminated by the methods that are currently used in sewage treatment. Substantial quantities of biosolids and livestock manure end up on agricultural land.

Biowaste is known to contain pathogenic bacteria such as Salmonella and other microorganisms that may be a health risk for both people and animals. The biosecurity risk associated with using digested residues as fertiliser is hard to assess but this risk can not be neglected.

# Comparison of humic acids from compost and peat as amendments of heavy metal polluted soils

Rafael Clemente and M. Pilar Bernal

*Department of Soil and Water Conservation and Organic Waste Management,  
Centro de Edafología y Biología Aplicada del Segura, CSIC.  
Apartado 164, 30100 Espinardo, Murcia, Spain. E-mail: rclemente@cebas.csic.es*

Organic matter is one of the main factors controlling the bioavailability of heavy metals in soils. The metal binding capacity of the humic acids and their low mobility in the soil make them metal-immobilising factors. The aim of this work is to study the effect of humic acids on the availability and distribution of soil heavy metals in an incubation experiment.

An acid soil (pH = 3.4) and a calcareous soil (pH = 7.7) from an ancient Pb-Zn mine area at La Unión (Murcia, SE Spain) were selected. The soils have high Zn and Pb concentrations (966 and 9229 mg kg<sup>-1</sup> respectively in the acid soil; 2602 and 1572 mg kg<sup>-1</sup> in the calcareous soil). The humic acids were isolated from a mature compost and a commercial *spaghnum* peat, by acidifying the NaOH extract and purification of the resulting precipitate

An incubation experiment (26 °C, 60 % water holding capacity) was carried out with both soils treated with humic acids from compost (HAC) and from peat (HAP) (1 g organic-C per 100 g soil), and using a control without amendment. After 2, 8 and 24 weeks, the soils were sampled and different fractions of heavy metals were determined by AAS after sequential extraction (McGrath and Cegarra, 1992. J. Soil Sci. 43, 313-321). The dynamic of C-mineralisation was determined by measuring the CO<sub>2</sub> evolved during the incubation time, trapping it in a NaOH solution.

The mineralisation of the humic acids, from both compost and peat, in the acid soil averaged 5.3 % of TOC with a constant mineralisation rate after 80 days of incubation. This result confirmed the high microbial stability of the humic acids from both materials. In the acid soil, humic acids caused the immobilisation of Zn and Pb from organic matter and EDTA-extractable fractions, shifting them to the non-available fraction, while Cu and Fe were slightly mobilised from the less available fractions to that linked with the organic matter. In the calcareous soil there were lesser effects. Initially Cu, Fe and Zn were solubilised from the resistant fractions to that linked with the organic matter. These effects could be due to the decrease in soil pH after HA addition to the calcareous soil, and after 6 months hardly any effect was observed. The use of organic materials with a high content of humic acids as soil amendments could well be successful in immobilising heavy metals in acid soils, whereas their use in calcareous soils is expected to be less effective and pH-dependent.

# **Problems connected with the EU-Animal By-product Regulations in the field of Environmental and Animal Hygiene**

**Reinhard Böhm**

*Universität Hohenheim, Institut für Umwelt- und Tierhygiene 460b,  
D- 70599 STUTTGART , boehm@uni-hohenheim.de*

The regulations (EC) No 1774/2002 of October 2002 is laying down health rules concerning animal by-products not intended for human consumption. According to the TSE-risk connected to their origin and utilization three different categories of Materials are defined in the legislation. The category 1 materials are connected with the highest TSE – risk while the category 3 materials which are in principle fit for human consumption have the lowest TSE-risk. Category 2 materials which shall be processed in biogas plants or in composting had to be sterilized in before at 133 °C/3bar/20 min. Category 3 materials may be processed in an biogas plant after pasteurization at 70 °C for 60 min having been crushed to a maximum particle size of 12 mm. Such material may also be composted if a minimum temperature in all material in the reactor is exposed to temperatures of 70 °C for 60 min. The question may arise if such materials are used as fertilizers on pasture land or on fields used for producing green fodder, if those measures are safe enough, because residuals infectivity may be ingested by susceptible animals under certain conditions. With respect to TSE- infectivity and other animal pathogens the utilization of sterilized category 2 material in aerobic and anaerobic treatment is safe enough, since more than 6 log of infectivity even in sporeforming conventional agents will be destroyed as well as at least 3 log of accidental or residual TSE-infectivity. The problem is with the category 3 materials. If they would contain accidental (eg. cross contamination) or residual infectivity the defined treatment processes will not eliminate them. Same applies for heat resistant viruses as parvo - viruses or calici – viruses. Experimental data will be given supporting this view and proposals for validation strategies for the treatment devices will be presented. The second complex of problems arises due to the given regulations for trading manure. Trade of unprocessed manure of species other than poultry or equidae is prohibited in principle (some exceptions are given). Especially poultry manure is critical from the point of view of Salmonella, the known problems with visceral botulism related to spreading of poultry manure and in the view of critical situation of introducing influenza viruses into the biocoenosis. Some details on epidemiological pathways related to such manure will be given.

# **Survival of pathogenic bacteria and parasites in stored sewage sludge**

**Ingela Berggren, Mats Johansson and Ann Albihn**

*National Veterinary Institute (SVA), Department of Disease Control and Biosecurity, SE-751 89 Uppsala, Sweden. [ingela.berggren@sva.se](mailto:ingela.berggren@sva.se). [mats.johansson@sva.se](mailto:mats.johansson@sva.se), [ann.albihn@sva.se](mailto:ann.albihn@sva.se)*

Sedimentation and mesophilic digestion of sludge are conventional treatments that cause a limited reduction of pathogens. These methods do not result in a hygienically safe sludge and pathogens, such as *Salmonella* spp., may therefore still be present in the final sludge products. At the National Veterinary Institute (SVA), some research projects are focused on hygienic aspects of recirculation of biological wastes back to agriculture and food production. Long-term storage of sludge after conventional treatment may result in a sludge product with better hygienic standard. However, international studies have revealed that storage alone is an insufficient method for hygienisation of sludge. In 2003 we initiated a study to assess the bacterial die-off during long-term sewage storage under Swedish climate conditions. The stored sludge, conventionally treated, will be monitored for presence and quantity of *Salmonella*, faecal enterococci and coliform bacteria during one year. The temperature in the sludge piles will be continuously recorded with data loggers. Furthermore, the effect of sludge temperature on the reduction rate of *Salmonella* and indicator bacteria will be intensively studied in a specific laboratory trial. Fresh sewage sludge, free from natural contamination of *Salmonella* spp. will be inoculated with *Salmonella Typhimurium*, previously isolated from Swedish sewage sludge. The sludge will also be provided with nylon bags containing *Ascaris suum* eggs. The sludge will be stored at three different temperatures (7, 14 and 21C°) and analysis will be performed regularly during a six-month period. The progress of these studies will be presented.

# **Heavy metal fluxes in livestock farming and input reduction strategies**

**Ute Schultheiß, Helmut Döhler, Ursula Roth and Henning Eckel**

*Association for Technology and Structures in Agriculture (KTBL),  
Bartningstrasse 49, 64289 Darmstadt, Germany  
email: u.schultheiss@ktbl.de*

Animal manures contain heavy metals as well as nutrients and trace elements. The spreading of animal manures which are contaminated with heavy metals can lead to an accumulation of these elements in agricultural soils. The objective of this research project was to record the flows of heavy metals in animal production systems and to develop a strategy for reducing heavy metal inputs into animal manures.

Twenty farms which practice animal husbandry in various regions of Germany were selected for investigation. The selection took into account different husbandry systems and farm sizes. For the calculation of the stable balances the inputs of heavy metals from feed stuffs and other farm sources (e.g. bedding, hoof disinfectants, medication, water) were contrasted to the outputs in the animal manures and animal products.

Apart from hoof disinfectants, feed stuffs and feed additives are the main input sources of heavy metals in animal manure. Purchased supplementary and complete feeds often show higher heavy metal contents than homegrown feeds. This is because feed stuffs are supplemented with trace elements. However, despite their usually low heavy metal content, homegrown feed stuffs are the main source of heavy metals in manure on dairy farms due to their widespread use.

Animal manures from pig production, and also partly from poultry production, showed higher concentrations of copper and zinc than manures from cattle production.

The stable balances for different livestock farms with cattle and pig production showed that the output levels of chromium, lead and zinc with animal products and manure exceeded their input levels. This was also the case for nickel and copper in pig production.

For minimising heavy metal contents in animal manures the reduction in the supplementary levels of copper and zinc in feed stuffs is being discussed. By approaching the copper and zinc dosages to the recommendations of scientific societies clear reductions would be possible. Further options are offered by other element rich inputs, e.g. hoof disinfectants in cattle production. Thus, evaluations and mitigation strategies need to tackle purchased feed stuffs which are already supplemented with trace elements and other element rich inputs.

# Effects of long-term applications of liquid manures to grassland on soil salinity

Paul N. C. Murphy<sup>1</sup>, R. James Stevens<sup>1,2</sup> and Peter Christie<sup>1,2</sup>

<sup>1</sup>*Agricultural and Environmental Science Department, Queen's University Belfast, and*  
<sup>2</sup>*Department of Agriculture and Rural Development for Northern Ireland, Newforge Lane, Belfast BT9 5PX, UK. E-mail: peter.christie@dardni.gov.uk*

Long-term application of liquid manures to grassland may alter soil chemical and physical properties. Soil salinity may increase, damaging soil structure and altering soil hydraulic properties. The aim of this study was to test whether long-term liquid manure applications alter soil salinity.

In February 2002, composite soil samples (0-5 cm depth) were collected from a long-term field experiment. There were eight treatments: plots receiving mineral fertilizer (FE), neither fertilizer nor manure (UF), pig slurry at 50 (LP), 100 (MP) or 200 (HP) m<sup>3</sup> ha<sup>-1</sup> year<sup>-1</sup> or cow slurry at the same three rates (LC, MC, HC). Soil samples were analysed for exchangeable Na and cation exchange capacity. Exchangeable sodium percentage (ESP) was calculated and used as a salinity indicator:

$$\text{ESP} = \text{Na}_{\text{ex}} / \text{CEC} \times 100,$$

where Na<sub>ex</sub> is exchangeable Na and CEC is cation exchange capacity (both meq/100 g).

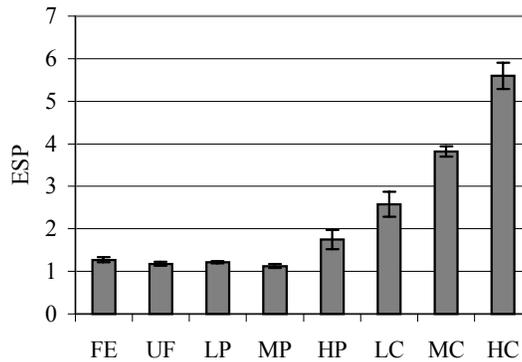


Fig. 1. Mean ESP for all treatments.

Mean ESP was significantly different among treatments and was increased by the medium and high application rates of pig slurry and all rates of cow slurry compared to the fertilized and unfertilized controls (Fig. 1). Increased ESP could affect soil physical properties and lead to decreased hydraulic conductivity and infiltration capacity. This may promote runoff, a potentially important P loss pathway. The capacity of the soil to sorb P anions may also be decreased by the increase in monovalent cations. This combination of physical and chemical factors may affect P loss from soils to surface waters.

## **Heavy metals transfer from soil to rapeseed oil**

**Corinne Bernhard-Bitaud<sup>(1)</sup>, Bruno Bourrie<sup>(1)</sup>, Jacques Evrard<sup>(2)</sup>, Pierre Burghart<sup>(2)</sup>, Xavier Pages<sup>(3)</sup>, Florence Lacoste<sup>(3)</sup>**

*(1) SADEF-Recherche Pôle d'Aspach, France, (2) CETIOM, France, (3) ITERG, France*

Transfer coefficients of heavy metals (brought in non toxic doses for rape crop) study showed that chromium, mercury and lead are very little transferred while cadmium, copper, zinc and nickel are much more transferred in rapeseeds. Cadmium and copper, often found in urban sludges, involve health (Cd) and technological (Cu) risks. So a rape crop has been conducted in field on an acidic loamy soil previously treated with either a Cd-rich urban sludge, or a Cu-rich urban sludge, or Cu salt. Higher treatments for copper and cadmium corresponded to the maximal level of metal inputs authorized for ten years by French regulations on sludge uses in agriculture (1998). High inputs didn't increased seed Cu-content while Cd-rich urban sludge led to higher seed Cd-content. Seeds have been crushed and refined in quasi-industrial pilots. Trace metals observed in seeds are almost exclusively transferred to meals after seed crushing. After the refining step, no risk for human health could be identified regarding the rapeseed oil in these conditions of cropping and industrial process. The rapeseed oil characteristics fitted with the hardest industrial specifications and even meals Cu and Cd-contents fitted with feed regulations.

This research was financially supported by ADEME (Agence de l'Environnement et de la Maîtrise de l'Energie, contract n°9775035)

## **Revising german biowaste ordinance hygiene rules for biogas-plants in accordance with Regulation (EC) No. 1774/2002**

**Susanne Klages\*, Eleonore Marciniscyn\*\*, Werner Philipp\*\*\*, Markus Schwab\***

*\*) Association for Technology and Structures in Agriculture (KTBL),  
Bartningstrasse 49, 64289 Darmstadt, Germany, email: s.klages@ktbl.de*

*\*\*\*) PlanCoTec, Karlsbrunnenstr. 11b, 37297 Neu-Eichenberg, Germany*

*\*\*\*\*) Institute for Environmental and Animal Hygiene,  
University of Hohenheim, 70593 Stuttgart, Germany*

The number of biogas-plants in Germany has reached approximately 2.000 in 2003, most of them being co-digestion-plants treating liquid manure, organic residues and renewable primary products. The German biowaste ordinance was rendered in 1998 to set up, among other items, hygiene standards for biowaste treated in biogas-plants. When passing the ordinance, the Bundesrat of the German Federal Republic demanded to scientifically inspect hygiene rules for anaerobic digestion, as, at that time, few research had been done on this field. Since May 2003, Regulation EC No 1774/2002 (Health rules for animal by-products not intended for human consumption), which has to be applied directly in the member states, supplies a frame for future national legislation.

Two research projects, conducted by PlanCoTec, a project work in Neu-Eichenberg and the Institute for Environmental and Animal Hygiene, University of Stuttgart-Hohenheim, examined the sanitation effect of sterilisation units (70°C), of thermophilic ( $\geq 55^\circ\text{C}$ ) and mesophilic working anaerobic digestion plants. Industrial plants as well as farm scale plants treating biowaste were checked. Test methods layed down in the biowaste ordinance, especially a start-up test consisting of direct (test organismes: Salmonella Senftenberg, Tobacco Mosaic Virus, Plasmodiophora brassiceae, tomato seeds) and indirect process test (temperature, treatment period) as well as a product test (test organisms: Salmonella, germinating seeds and propagules) were examined. On behalf of the Federal Ministry of Environment, the Association for Technology and Structures in Agriculture (KTBL) accompanies both projects and for this reason established an expert committee in order to draft a proposition for optimising national hygiene rules for biogas-plants treating biowaste. These new rules have to be in line with EU-regulations.

Biogas-plants without sterilisation unit might not meet present EU standards (Enterobacteriaceae, Salmonellen). A thermophilic operation of plants is up to now not accepted in Regulation EC No 1774/2002. The start-up test according to the german biowaste ordinance has turned out not to be practicable for biogas-plants. This is mostly due to the fact that the conditions layed down in the direct process test are not sufficiently standardised and, up to now, most biogas-plants are not designed for running this test. Furthermore, the high tenacity of the Tobacco-Mosaic-Virus under anaerobic conditions implies test conditions which do not represent standard treatment. Future national hygiene rules will therefore focus on a optimisation of phytohygienic rules in accordance with treatment conditions and test modes of EU-legislation.

# Assisted natural remediation of trace element polluted soils

Pilar Burgos, Alfredo Pérez de Mora, Engracia Madejón and Francisco Cabrera.

*Instituto de Recursos Naturales y Agrobiología de Sevilla (IRNAS-CSIC). Apartado 1052. 41080. pburgos@irnase.csic.es*

Soils affected by the Aznalcóllar spill present pollution by trace elements and a considerable deterioration of the physical, chemical and biological properties. The application of amendments and the establishment of plants are techniques of *in situ* metal immobilization, called Assisted Natural Remediation techniques (ANR), that play an important role in the restoration of polluted soils. This paper studies the effects of different amendments on soil quality and on the bioavailability of the polluting elements after two successive applications.

The experiment was carried out in 24 containers placed outdoors, filled with the contaminated soil (the first 25 cm layer) (pH 3.49, As 120 mg kg<sup>-1</sup>, Cd 2.44 mg kg<sup>-1</sup>, Cu 78 mg kg<sup>-1</sup>, Mn 654 mg kg<sup>-1</sup>, Pb 201 mg kg<sup>-1</sup> and Zn 226 mg kg<sup>-1</sup>). Four organic amendments (MWC, a municipal compost, BC, a biosolid compost, LEO, a leonardite and LIT, a litter) and an inorganic amendment (SL, sugar beet lime) were mixed with the top 10 cm soil at doses of 100 t ha<sup>-1</sup> in the first year and 50 t ha<sup>-1</sup> in the second one. Unamended soil was used as control. Four replicates per treatment were randomly established in a complete block design. The containers were sown with *Agrostis* and irrigated when necessary.

Amendments increased soil pH and TOC (Table 1) and decreased the concentrations of Cl<sub>2</sub>Ca-soluble Cu, Mn and Zn (Table 1). EDTA-extractable fractions of Zn, Pb, Mn, Cu, Cd and As in soils amended with MWC and BC were significantly higher than in the control (Table 1). However, plants from soils amended with the materials showed, in general, lower trace element concentrations than the control plants. In this case a negative relationship between plant extraction and metal EDTA extractable fraction were found. Nevertheless, the values of Cl<sub>2</sub>Ca-soluble and EDTA-extractable trace elements in soil after two years of experiment in all treatments (amended and control) decreased in comparison with the values of the original soil, displaying the importance of the plant cover in heavy metal stabilization.

**Table 1.** pH values and soluble (0.01M CaCl<sub>2</sub>) and available (EDTA) concentration of As and heavy metals in soils treated with the different amendments after two years of experimentation.

Treat.	pH	Cu	Mn	Zn	As	Cd	Cu	Mn	Pb	Zn
		CaCl <sub>2</sub>	CaCl <sub>2</sub>	CaCl <sub>2</sub>	EDTA	EDTA	EDTA	EDTA	EDTA	EDTA
(mg kg <sup>-1</sup> )										
<b>Control</b>	4.63 a	2.56 c	28.8 bc	30.6 c	2.37 a	0.18 a	31.3 ab	42.0 a	1.60 a	38.3 a
<b>MWC</b>	6.69 bc	1.01 ab	3.24 a	1.66 a	5.78 bc	0.61 b	49.3 c	219 bc	12.6 b	89.3 bc
<b>BC</b>	6.73 b	0.79 ab	1.51 a	1.75 a	7.29 c	0.56 b	37.7 b	238 c	6.60 ab	110 c
<b>LEO</b>	4.73 a	0.30 a	42.0 c	25.0 bc	1.71 a	0.44 ab	29.0 a	124 b	2.42 a	91.3 c
<b>LIT</b>	5.40 a	0.65 ab	11.5 b	10.3 ab	1.69 a	0.39 ab	32.8 ab	105 ab	3.05 a	63.1 b
<b>SL</b>	7.49 c	0.71 b	0.94 a	0.67 a	4.46 b	0.50 ab	25.1 a	39.7 a	1.47 a	48.4 ab

# **Molecular analysis of the microbial community dynamics in pig slurry during storage and after soil application**

**Pascal Peu, Patrick Dabert, Anne-Marie Poucher, Jean-Jacques Godon Et  
Philippe Delgenes**

*Cemagref, Livestock and Municipal Waste Management Unit. 17 avenue de Cucillé. CS  
64427 35044 Rennes Cedex France. e-mail : pascal.peu@cemagref.fr*

The evolution of the dominant microbial community of a pig slurry was followed in a pig farm during 6 months. Sampling was carried out on all the different management steps of effluent : faeces, storage tank, lagoon of storage and soil before and after slurry spreading. Total DNA of these various samples were extracted and analyzed by PCR-SSCP, in particular for the *archaea* and *bacteria* domain and for specific bacterial groups. This study shows first a relative stability in time of the microbial populations met in the stored pig slurry. However, migration of this effluent through different environments (e.g. digestive tract - to storage tank) modify the microbial community probably to the profit of better adapted species. Lastly, micro-organisms present in the effluent could not be detected any longer in soil after spreading, using the SSCP technique.

The dominant *archaea* of the flora of faeces and pig slurry were identified. They belong to genus *Methanosphaera*, *Methanobrevibacter* and *Methanogenium*, all hydrogenotrophic *archaea* methanogens. No aceticlastic *archaea* methanogen were found, in spite of the strong acetate content measured in pig slurry. SSCP *bacteria* profiles obtained reflect a large bacterial diversity where 9 dominant phylotypes can be characterized. Due to the complexity of obtained profiles, bacteria genders were grouped by targeting three dominant bacterial groups such as *Clostridiaceae*, *Bacillus-Streptococcus-Lactobacillus* and *Cytophaga-Flexibacter-Bacteroides*. Identification of dominant phylotypes was carried out for these 3 groups. The majority of identified phylotypes are close to uncultivated bacteria. With these results, identification of 5 of the 9 dominant bacterial phylotypes of pig slurry was carried out. Bacteria mostly represented in this ecosystem corresponds to bacteria not cultivated yet closely related to *Clostridium butyricum* and four other are close to uncultivated bacteria belonging to genus *Prevotellaceae*, *Bacteroidaceae* and *Streptococcaceae*.

# **Adsorption of phenoxyacid herbicides by soil from North of Morocco amended with urban sewage sludge**

**Jamila Haouari<sup>1</sup>, Abdelmalek Dahchour<sup>2</sup>, Mohammed Alaoui-ElBelghiti<sup>1</sup> and Aránzazu Peña<sup>3</sup>**

<sup>1</sup>*Département. de Chimie, Fac. des Science de Rabats, Laboratoire de Chimi-Physique, BP 1014 RABAT-Agdal (Morocco).*

<sup>2</sup>*Institut Agronomique et Vétérinaire Hassan II. BP 6202 RABAT-Instituts (Morocco).*

<sup>3</sup>*Dpto. de Ciencias de la Tierra y Química Ambiental. Estación Experimental del Zaidín (CSIC). Profesor Albareda, 1. 18008 – Granada (Spain)*

The use of pesticides in Moroccan agriculture has been steadily increasing in the recent decade to cover different crops. Changes occurring in the climate have deeded Morocco in chronic semi-arid area, with reduced rainfall averages and prolonged hot periods. This could affect the behaviour of pesticides in the soil and lead to different ultimate situations in terms of persistence, mobility and degradation, mainly after severe storms or abundant irrigation. Therefore, three different soils from the Gharb area, a large agricultural zone at the north of Rabat (Morocco), have been selected to study their interaction with herbicides. In an attempt to promote soil sorption and retard migration of some herbicides, urban sewage sludges, or biosolids, have been applied to the soil.

Dewatered urban sewage sludge, from a municipal wastewater facility in Granada (Spain), was used for soil amendment. With respect to pesticides, phenoxyacid herbicides (2,4-D, mecoprop and dichlorprop) together with salicylic acid, have been selected. Adsorption kinetics and isotherms of the compounds in the three soils, amended or not with 2, 4 and 10% urban sewage sludge, have been carried out with the batch equilibration procedure. Analysis of the herbicides was performed by HPLC with DAD.

The data were fitted to the Freundlich equation in all cases. For the three herbicides only a slight adsorption, ranked as follows, 2,4-D > MCPP  $\approx$  DCP, was found on unamended soils. The addition of biosolids to the soils had a visible effect on herbicide adsorption at the highest dosage (10%) which is five times higher than permitted. At the recommended dosage (2%) a non-significant increase in adsorption was measured for the herbicides.

Similar results with ionisable compounds have been attributed to the repulsion of the anionic molecules with the negatively charged surfaces.

This study has been funded by NATO Science Programme (EST.CLG.978624)

# **Adsorption of sulfonylurea herbicides by soil amended with different olive oil mill wastes**

**Laura Delgado and Aránzazu Peña**

*Dpto. de Ciencias de la Tierra y Química Ambiental. Estación Experimental del Zaidín (CSIC). Profesor Albareda, 1. 18008 – Granada (Spain)*

Agricultural by-products, along with other waste disposal amendments of high organic matter (OM) content, are applied to soils with low OM content to enhance their fertility and improve their physicochemical properties. Moreover, the addition of exogenous material to soil (peat, manure, sewage sludge, compost, etc), has been extensively used in the last years to modify pesticide fate in the environment. The olive tree (*Olea europaea* L.), an important and characteristic culture of the Mediterranean area, is mainly used to produce olive oil. The extraction of olive oil generates some by-products with potential application in crop management, which can be used without further treatment (wet olive cake) or by modifying them with composting or vermicomposting processes.

Adsorption kinetics and isotherms of three sulfonylurea herbicides (chlorsulfuron, bensulfuron-methyl and prosulfuron) have been carried out with the batch equilibration procedure in a soil proceeding from Granada (Spain) amended or not with wet olive cake (CA), with its compost (C) and its vermicompost (V). Analysis of the herbicides was performed by HPLC with diode-array detection.

Chlorsulfuron and prosulfuron were only weakly retained by the soil and the adsorption was not enhanced by the presence of the amendments. Increased sorption was encountered for bensulfuron-methyl, especially when wet olive cake (CA) was added to the soil. These results agree with previous reports on ionisable pesticides, for which no relationship between sorption and soil organic matter content has been found, a fact explained by the repulsion between the negative charge of the molecules and the net negative charge of organic matter and clays.

This study takes part of a research Project (CAO001-007) funded by Junta de Andalucía.

## Evolution of biochemical parameters in the suppression of the damping off caused by *Rhizoctonia solani* after the addition of sewage sludge compost.

**Yolanda Arribas-Santamaría, Belén Alonso-Núñez, D. Javier López-Robles,  
Carlos Rad and Salvador González-Carcedo**

*Edaphology and Agricultural Sciences. Faculty of Sciences. University of Burgos. E-09001  
Burgos. Castile (Spain). yasga@ubu.es*

Composts have long been recognized to provide a degree of control of diseases caused by soil-borne plant pathogens and the use of compost for disease control is of increasing interest. Many of the mechanisms involved in control of fungal pathogenic species are linked with the increase of biological activity due to organic matter addition, the change in microbial abundance and specific antagonistic activities of microorganisms presents in mature composts. In this work, the suppression of sugar-beet damping off caused by *Rhizoctonia solani* is analyzed in relation to the evolution of their biochemical properties during an incubation experiment of mixtures of infected soil and increasing amounts of sewage sludge compost.

A calcareous clay loam soil, with low organic matter content (1.85%) was incubated over a period of fifteen weeks at 25 °C and 60% WHC, in which periodically the original soil, the soil infected with a culture of *R. solani* and the infected soil with 0.1, 0.5 and 1% mature sewage sludge compost, were analyzed in terms of biological activity: INT-dehydrogenase activity (DhA), FDA-hydrolase (FDAH), arginine ammonification (ArgA), alkaline phosphatase (AlcPA) and  $\beta$ -D-glucosidase activities ( $\beta$ GA). In addition, at the beginning and at the end of the incubation, the Zucchini's germination test with *Lepidium sativum* seeds and the suppression of the fungal pathogenic effect were assayed with sugar beet seeds which were grown during 45 days of incubation in a climatic chamber under controlled conditions of temperature, light and humidity. The activity of the fungal pathogen was increased as a consequence of the incubation, mainly in the presence of the lowest amount of compost; however, increasing quantities of compost which had different initial responses to sugar beet survival, had a final positive effect in the number of viable plants (Table 1). Enzymatic activities showed different evolution during the incubation time. Endocellular activities, such as DhA and FDAH, decreased during the incubation time with rapid responses to the initial addition of organic matter or water replacement. More stable activities by extracellular immobilization, such as AlcPA or  $\beta$ GA showed an increase after 105 days of incubation, mainly in the highest doses of compost, which could provide an important source of nutrients for the microbial antagonistic groups of the pathogen.

**Table 1.** Percentage of plant survival in the original soil and the infected soil with or without different amounts of compost at the beginning and at the end of 15 weeks incubation at 25°C and 60% WHC.

Sample	S <sub>o</sub> <sup>a</sup>	S <sub>p</sub> <sup>a</sup>	S <sub>p</sub> + 0.1% C <sup>a</sup>	S <sub>p</sub> + 0.5% C <sup>a</sup>	S <sub>p</sub> + 1% C <sup>a</sup>
% Survival Initial Sample	65.85	55.26	54.35	25.53	86.05
% Survival Incubated Sample	66.67	39.47	21.95	61.82	67.44
% G.I. Initial Sample <sup>b</sup>	1.26	0.73	0.90	1.10	1.14
% G.I. Incubated Sample <sup>b</sup>	0.83	1.04	0.90	0.82	0.88

<sup>a</sup>S<sub>o</sub>: original soil, S<sub>p</sub>: soil infected with *R. solani*, S<sub>p</sub> + C: soil infected with different amounts of compost.

<sup>b</sup>G.I.: Germination Index.

## **Heavy metals biosorption by compost-associated microorganisms: practical applications**

**López, M.J., Vargas-García, M.C., Suárez-Estrella, F., Guisado, G. and  
Moreno, J.**

*Area de Microbiología. Dpto. Biología Aplicada. CITE II-B. Universidad de Almería.04120  
Almería. mllopez@ual.es*

Industry, mining and agricultural activities have led to large scale contamination of the environment with toxic heavy metals. Several treatments are available for metal remediation, but most are expensive to apply and lack specificity. Biosorption is an approach that offers many advantages including highly selective removal of toxic metals coupled with considerable operational flexibility. This method implies the use of natural substrates such as agricultural wastes or peat and involves physicochemical together with biological mechanisms for metal decontamination.

The aim of this work was to study the bioremediation of a mixture of heavy metals in water using compost and horticultural plant residues. We also analysed the involvement of natural microbiota in metals removal, as well as the effect of several nutritional and environmental conditions on this microbial activity. We found that physicochemical sorption occurs mainly during first hours of contact between contaminated water and substrate, while after three to 24 hours contact, detoxification is mainly mediated by microorganisms which colonize material. This microbial activity may be increased by means of adjusting at adequate values factors that improve microbial growth such as nutrients, pH, oxygen and temperature. After selection of optimal conditions we were able to increase heavy metal elimination up to 80%.

According to these results, compost or plant waste residues may be used for heavy metal remediation and efficacy of process can be improved by optimizing factors that increase microbial growth.

## **Management Sewage Sludge Thermal Drying Amended Soil**

**Delgado <sup>1</sup> M., Porcel M.A.<sup>1</sup>, Miralles De Imperial R.<sup>1</sup>, Beltrán M<sup>a</sup> <sup>1</sup>,  
Beringola M<sup>a</sup> L.<sup>1</sup> Martín J.V.<sup>1</sup>**

*<sup>1</sup>Department Enviroment, INIA Apdo 8111. 28080 Madrid. Spain. E mail: delgado@inia.es*

Increasing sewage sludge production (Directive 91/271/CE) and use restrictions due the landfill directive (99/32/EC) give priority to the application of sewage sludge to the soil. The chemical composition of sewage sludges is of great importance when developing recommendations for the rates of sludge applications on agricultural land. At the present time, recommendations for sludge applications rates on land are based on the fertilizer value (N, P and K) and on the concentrations of trace metals present in sludge. The metals of primary concern are Zn, Cu, Pb, Ni and Cd which, when applied to soils in excessive amounts, may reduce plant yields or impair the quality of food or fiber produced.

Studies were conducted to determine using of an organic residue (sewage sludge thermal drying) during two years (2002-20023), to study effects of sewage compost on crop yield and chemical properties of soil under field condition. Productivity studies showed the great growth is obtained in mixed II treatment (10000 kg/ha sewage sludge thermal drying plus 350 kg/ha urea) with 20% more than mineral fertilization and followed mixed I (8000 kg/ha sewage sludge thermal drying plus 350 kg/ha urea) with 10% more than mineral fertilization also. No toxic effects arising from the heavy metals in the plant were observed. Moreover heavy metals concentration's in soil are below Spanish and European legal limits.

Key words: Sewage sludge, reusing, fertilization, production, heavy metals, Zea mays L. Variety Juanita.

# Heavy metal distribution in sewage sludge-treated soil profiles

**M.J. Sánchez-Martín, M. García-Delgado, L.F. Lorenzo and  
M. Sánchez-Camazano**

*Instituto de Recursos Naturales y Agrobiología de Salamanca, CSIC.  
Apdo 257, 37071 Salamanca, Spain. Email: mjesussm@usal.es*

The production of sewage sludges has increased in Spain to a large extent as a result of the increase in sewage treatment plants constructed in recent years. As is known, these sludges have a high content in organic matter and appropriate levels of nutrients, making them potentially useful as soil amendment. However, the presence of heavy metals in such materials is worrying from the environmental point of view since if their availability is high they may give rise to contamination within the food chain or may pollute ground waters. In this sense, the increase in the total load of heavy metals in soils treated successively with sewage sludges and their mobility in the soil profile are of great interest.

In this work we studied the total content of heavy metals (Cd, Cu, Cr, Ni, Pb and Zn) in a soil treated with different sludges and the distribution of these metals in the soil profile (50 cm) as a function of the type and dose of sludge added, of the residence time of the sludge in the soil, and of the successive addition of sludges to the soil. The study was carried out on experimental plots prepared on a sandy loamy soil. Two sludges, subjected to different treatments (W1 and W2), from sewage treatment plants of different localities were added at doses of 40t/ha and 100t/ha.

The concentrations of metals in the soil of the control plots were low at the surface and remained almost constant in down the soil profile. In the amended soils, only the concentrations of Zn, Cu, Cd and Pb in the soil treated with W1, and of Zn and Cu in the soil treated with W2 increased slightly with respect to the control soil. The most significant increase was found in the upper layer of the soil profile (0-20 cm) after 1 year of treatment with the highest doses of the sludges applied. The results reveal that almost no redistribution of the metals present in the sludges in the profile of the amended soils had taken place, indicating that under the experimental conditions used and for the two sludges studied no vertical movement of the metals present in the sludges had occurred from the zone of application of these materials.

## **Risks of heavy metal transfer to *Beta vulgaris* cultivated in a contaminated soil and amended with organic wastes**

**Rafael Clemente<sup>1</sup>, José L. Martínez<sup>1</sup>, Concepción Paredes<sup>2</sup> and M. Pilar Bernal<sup>1</sup>**

<sup>1</sup>*Department of Soil and Water Conservation and Organic Waste Management. Centro de Edafología y Biología Aplicada del Segura, CSIC. Campus Universitario de Espinardo, Apartado 164, 30100 Espinardo, Murcia, Spain. E-mail: pbernal@cebas.csic.es;*

<sup>2</sup>*Department of Agrochemistry and Environment, Miguel Hernandez University, EPSO, Ctra Beniel Km 3.2, Orihuela (Alicante), Spain*

The mining activity carried out in La Unión (Murcia) since Roman times has transformed completely the landscape of the mine site and adjacent land (24.55 km<sup>2</sup>), due to the accumulation of metalliferous mine wastes. The transport of fine particles by wind (dry deposition) has caused heavy metal pollution of agricultural soils in nearby areas.

This work reports the effect of two organic wastes (cow manure and olive husk) on the bioavailability of heavy metals in a soil affected by the mining activity. A field experiment, with nine plots of 6 m<sup>2</sup> each, was designed in a soil with 2380 mg kg<sup>-1</sup> of total-Zn, 4070 mg kg<sup>-1</sup> of total-Pb, 35 mg kg<sup>-1</sup> of total-Cu, 3 mg kg<sup>-1</sup> of total-Cd, and 27 mg kg<sup>-1</sup> of total-Ni. Three treatments, in a randomised design with three replicates per treatment, were established in experimental plots of 2×3 m<sup>2</sup>. The treatments were: fresh cow manure, olive husk, (both added at a rate of 30 kg per plot), and inorganic fertiliser N:P:K (15:15:15) at a rate of 0.8 kg per plot. Five weeks after waste application, 48 plantlets of *Beta vulgaris* var. Nomonta were planted in each plot. Two harvests were performed; two and three months from planting. The soil was sampled before planting and after the second harvest. Total heavy metals were analysed in plant material, and soils were analysed for available heavy metals (DTPA-extractable), and pH, electrical conductivity, organic carbon and cation exchange capacity.

The results indicate that the cow manure decreased the heavy metal bioavailability in soil, and their concentrations in plants, leading to the highest plant biomass production. However, olive husks increased the availability of metals, mainly Pb, Zn and Mn, which produced phytotoxicity. The decrease in soil pH caused by the olive husks was the main factor responsible for the increase in metal solubility and therefore plant metal uptake. The use of cow manure can be recommended for re-vegetation of soils contaminated by heavy metals.

## **Total and faecal coliform bacteria persistence in a pig slurry amended soil**

**Ana Belen Rufete, Maria Dolores Perez-Murcia, Aurelia Perez-Espinosa, Raul Moral, Joaquin Moreno-Caselles, Concepcion Paredes**

*Department of Agrochemistry and Environment, Miguel Hernandez University, EPS-Orihuela, Ctra Beniel Km 3.2, 03312-Orihuela (Alicante), Spain.*

Carbon and nutrients recycling usually favours the use of several raw wastes with a wide range of characteristics and heterogeneous nature in agricultural soils. Pig slurries are a suitable source of nutrients for the soil-plant system. However, the input of exogenous microorganisms from the pig slurry could interfere the biogeochemistry of the amended soils and also be a pathway for a biological hazard into the food chain. Although different authors reported that the survival time of coliforms in soils is relatively short (1-2 months), a regrowth of these pathogens into the amended soils is possible. In addition, Gibbs et al. (1997) concluded that soil amended with biosolids could not be considered free from pathogens for at least one year following amendment.

The aim of this experiment was to study the effect of the application of pig slurry on potential pathogens (total and faecal coliform bacteria) presence in a Typic Xerofluent soil with a high intensive agricultural use. The experiment was conducted in field conditions, in the Segura river valley in the South East of Spain. Four treatments, in a fully randomised design with three replicates per treatment, were established in experimental plots of 16 m<sup>2</sup> each one, with a 0.5 m distance between plots. Fertiliser treatments were: MF, mineral fertiliser (with a complex 16 N-16 P<sub>2</sub>O<sub>5</sub>-16 K<sub>2</sub>O) equivalent to 150 kg N ha<sup>-1</sup>; PSF, organic fertilisation with swine manure slurry, at two different rates (supplying 150 kg N ha<sup>-1</sup>, and 210 kg N ha<sup>-1</sup>), and C, a control treatment without fertilisation. Samplings at 0, 5, 13, 25, 36, 50, 64, 88, 109, 175 and 295 days after the treatment applications were carried out.

A high spatial and temporary variability was detected, probably due to the high number of parameters that affect soil microorganisms. Total and faecal coliform bacteria presence was detected in control soils. In most cases, an increase in the pathogen bacteria content was observed in the soils amended with mineral fertiliser compared to control soils. However, the pig slurry amendment induced the highest initial and also persistent presence of total and faecal coliform bacteria. In general, total coliform bacteria were between 2-3 log<sub>10</sub> units higher than the faecal ones. Mostly, a decreasing tendency with time was observed in both pathogens in all the treatments.

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## **Detection of intestinal parasites in pig slurries collected from farms in Alicante Province (Spain)**

**Fernando Bornay-Llinares, Luis Navarro-i-Martinez, Fuensanta Garcia-Orenes, Hector Araez, Maria Dolores Perez-Murcia and Raul Moral**

*Department of Agrochemistry and Environment. University Miguel Hernández de Elche (Alicante) Spain. E-mail: f.bornay@umh.es*

The application of raw livestock wastes in agricultural soils is one of the most extended practices for residue management. However, there are diverse components in their composition, especially pathogens, heavy metals and salinity, which are potentially dangerous for the environment and for man. Waterborne transmission of intestinal parasites has been linked to domestic livestock and farming practices. As an example, *Cryptosporidium* sp. robust oocysts can survive for long periods outside the host, particularly in moist environments. The aim of this study was to investigate the presence of intestinal parasites in pig slurries from several piggeries in Alicante (Spain).

Pig slurries were collected in five high intensive pig farms (A-E) and sampled in each farm from the pits depending on the production cycle (gestating sows, farrowing sows, weaners, finishers). 25-l slurry samples were collected, after a mechanical homogenisation of the whole volume of the pit, when aged 30-60 days and immediately analysed in the laboratory. Samples were concentrated either through zinc sulphate flotation or by formalin-ethyl acetate sedimentation methods. Parasitological examination was performed by optical microscopy. Detection of *Cryptosporidium* sp. was performed using conventional acid-fast stain and by DNA extraction and PCR amplification of the diagnostic region of the small subunit ribosomal RNA (SSUrRNA) gene using CPB-DIAGF and CPB-DIAGR primers. Intestinal parasites were observed in all farms studied. The distribution of the species is shown in table 1. Parasite viability studies are needed in order to assess the potential concern on animal and human health.

## Lifecycle of metals in composting of MSW

A. F. Chica \*; A. Rosal \*; M. A. Martín \*; J. E. Castillo \*\*; and F. Herrera \*\*

*\*Inorganic and Chemical Engineering Dpt. \*\*Agricultural Resources Sciences Dpt.  
Campus Rabanales (Edif. Marie Curie.) University of Cordoba. 14071-Córdoba. España  
Fax: +34 957 218625 e-mail: afchica@uco.es*

Although there are not still specific European regulations on biological management of solid waste, the 1999 guideline about rubbish dumps stipulate a progressive reduction in the accumulation of organic matter in them.

When facing this problem one of the possible options, which is probably the most recommended one in countries with a progressive impoverishment of organic matter of their soils, is the composting process.

Thus, in this work the composting process, in turned windrow, of the organic fraction of the municipal solid waste, selectively collected, will be analysed for three years.

The composting process and the refined compost obtained have been characterized. The results on the evolution of the pH, conductivity, C/N relation, P, organic matter and other important variables have been presented. Metals content is shown in Table 1.

Suitable compost for agricultural use is obtained. This compost was used in wheat, garlic, and pepper cultures. Metals content in soil and fruit have been analysed. No incidence of metals on fruit and soil was detected. Crops were found to be similar to those observed for a control test. However, requirements in metal contents are increasing in EU rules. So, this Municipal Solid Waste Compost made in Cordoba (Spanish low industrial city) will not be accepted for an agricultural use.

**Table 1.** Metals content in MSW compost and proposed limits.

	Cd (mg/Kg)	Cu (mg/Kg)	Cr (mg/Kg)	Ni (mg/Kg)	Pb (mg/Kg)	Zn (mg/Kg)
MSW fresh	4	47	104	98	120	90
Test 2000-01	4	276	30	50	165	415
Test 2001-02	3	252	57	57	120	579
Test 2002-03	2	373	84	64	144	603
Spanish Law 05/28/1998	10	450	400	120	300	1100
Ecolabel 2001/688/CE	1,5	75	140	50	140	300
B.T.B. 2draft Compost – 1	0,7	100	100	50	100	200
B.T.B. 2draft Compost – 2	1,5	150	150	75	150	400
B.T.B. 2draft Stabilised BW	5	600	600	150	500	1500

The authors wish to express their gratitude to JUNTA DE ANDALUCIA (Project ACC.2001-02 RNM-271) and to MCYT (Project REN2002-02602) for financial support.

# Raw organic materials origin and compost heavy metal contents

Marga López, Oscar Huerta, Jordi Redó and Montserrat Soliva

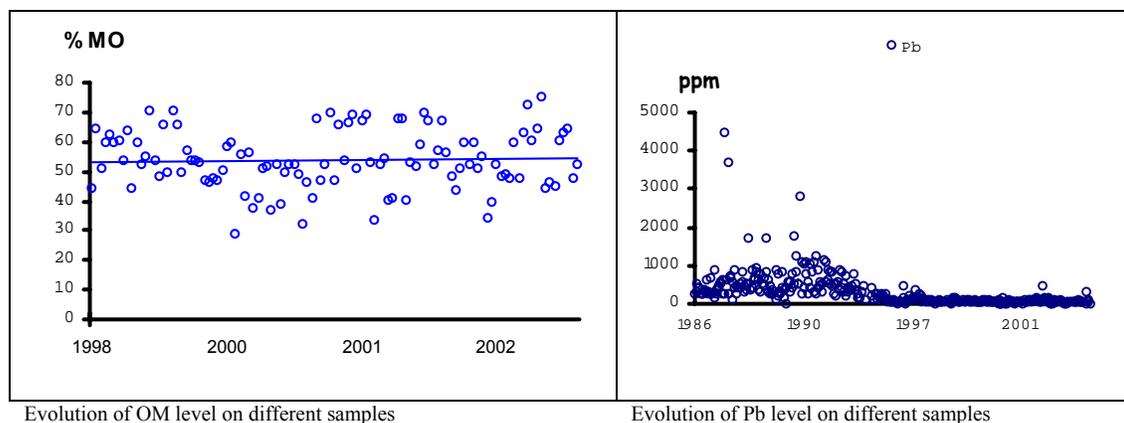
*Escola Superior d'Agricultura de Barcelona- UPC. Urgell, 187. 08036*

The heavy metal (HM) content of organic wastes (OW) is a parameter of great importance to prevent its transfer to soil-plant systems when OW are applied to agricultural soils, for that this parameter has been included in the current legislation for OW land reuse. The HM content could be considered in relation to different points of view: source, soil behaviour, soil-plant transference, its relationship to the organic matter (OM) or the nutrients levels and also according to the sort of OW and the treatment applied.

The working documents prepared by European Commission (on biological treatment of biowastes and to improve the present situation for sewage sludges) suggest expressing HM content according to the content of some agronomic parameters. For biological treatment of biowastes -compost, digestate and stabilized biowastes- in relation to a normalized values of 30 %OM; and for sewage sludges according to their phosphorus content.

The composition and characteristics of compost samples from different origin (mainly municipal waste) has been determined since 1996 to actuality. The total HM content was quantified by AA after dissolution of OW ashes (ignition at 470°C) in 3M HNO<sub>3</sub>.

The results show the HM content decrease with time for the municipal wastes, it is due to better mechanical separation systems in treatment plants or to the composting after OM separated collection.



In some cases, also a few environmental aspects related to OW composition are improved with time, but not the agronomic ones as OM content, N content or OM stability.

## **Volatile Fatty Acid Concentration and Inhibitory Effect upon some Pathogen Strains of Several Compost Extracts**

**Viuda-Martos, M.<sup>1</sup>; Gil-Conejero, A.<sup>1</sup>; Fernández-López, J.<sup>1</sup>; Sayas-Barberá, M.E.<sup>1</sup>; Marín-Martín, F.R.<sup>2</sup> and Pérez-Alvarez, J.A.<sup>1</sup>**

<sup>1</sup> *Food Technology Department. Universidad Miguel Hernández. Orihuela. Alicante, Spain*

<sup>2</sup> *Food Technology Department. Universidad Autónoma de Madrid. Madrid, Spain*

It is well known that compost could be used as soil enhancers, in this way compost could have effect not only in nutrients availability but also in the inhibition of some plant pathogens. The aim of this work was to analyse and determine the volatile fatty acid profile of different compost. Experimental compost (onion compost, green waste compost, spent mushroom compost and grape mark and olive press cake compost) were elaborated under UE fifth framework Recoveg project (Recycling Horticultural wastes to produce pathogen suppressant compost for sustainable vegetal crop production QLRT-01458). The volatile fatty acids (VFA) were extracted in acid media. VFA content was determined by gas chromatography (flame-ionization detector). The acid extracts were added to a PDA growth media to test its possible inhibitory effect over *Sclerotinia cepivorum*, *Aspergillus niger*, *Aspergillus flavus*, *Fusarium oxysporum*, *Phytophthora nicotinae*, and *Phytium ultimum*. The results showed that onion compost is the only sample that has the main VFA content (acetic, propionic and butyric acid), and valeric acid was not detected in any sample. Higher concentration on butyric acid only was found in spent mushroom compost. In green waste compost and grape mark wastes and in olive press cake compost, only trace of this compound were detected. This extract did not show any inhibitory effect on growth of the microbial strains analysed.

# **Effects of municipal waste composts on soil and crop heavy metals in grass cultivation in Finland**

**Tiina Tontti and Ritva Mäkelä-Kurtto**

*MTT Agrifood Research, Karilantie 2 A, FIN-50600 Mikkeli, tiina.tontti@mtt.fi*

*MTT Agrifood Research, FIN-31600 Jokioinen, ritva.makela-kurtto@mtt.fi*

The aim of this study was to investigate the effects of municipal waste composts on soil and crop heavy metals in grass cultivation in conventional and organic farming in Finland.

Two field experiments were conducted during 2000-2002, a grass experiment in conventional farming on fine sandy soil (pH 6,1) and red clover grass mixture in organic farming on fine sandy till (pH 6,5). The municipal waste composts studied were biowaste compost and biowaste+sewage sludge compost. They were applied in a single dose in spring 2000. The application rates respected the Finnish fertilisation recommendations for the two and four-year phosphorus needs of grass established with a cereal cover crop assuming that 75% of the total phosphorus in the composts was plant-available. No other nutrients were added in 2000. In 2001 and 2002, mineral nitrogen and potassium were supplied. The cereal cover crop was harvested in 2000 and 2-3 cuts of grass and mixture were collected in 2001 and 2002. The municipal waste composts were compared to mineral fertilisation in the conventional experiment and to cattle manure compost in the organic experiment. A split-plot experimental design with four replicates was used with a zero control (no fertilisation). The soils were sampled before any application and every autumn and spring thereafter. All the composts were sampled at the time of application and the crops at harvesting. The samples were analysed for nutrients and heavy metals.

The lowest metal input came from the mineral fertilisers and the second lowest from the cattle manure compost. During the 3-year experiment, the crops took up chromium and lead about 1-5%, cadmium and nickel 10-20% and copper and zinc 20-30% from the amounts added in the composts at the low application rate. At the high rate of application, metal uptake by the crops was a half of those at the low application rate, respectively. Metals added into the soil in composts had nearly no effects on the easily soluble concentrations of metals in soils.

# **Agronomic Use of Pig Slurry for Broccoli Production: Investigation of Pathogen Microorganisms**

**José L. Tortosa<sup>1</sup>, Ángel Faz<sup>1</sup>, Alfredo Palop<sup>2</sup>**

*<sup>1</sup>Área de Edafología y Química Agrícola. Departamento de Ciencia y Tecnología Agraria. Universidad Politécnica de Cartagena, Alfonso XIII, 52. 30203 Cartagena, Murcia-España. joseluis.tortosa@upct.es - angel.fazcano@upct.es*

*<sup>2</sup>Departamento de Ingeniería de Alimentos y del Equipamiento Agrícola. Universidad Politécnica de Cartagena, Alfonso XIII, 52. 30203 Cartagena, Murcia-España. alfredo.palop@upct.es*

In the past few years there has been an increasing public concern for the contamination of water, food and air by pathogens residing in manure, the subproducts of manure and bioaerosols.

The objective of this work was to study the microbial load of pig slurry, which is used as an organic fertilizer for broccoli cultivation and the possible relationship with the microbial content of the crop.

The research was carried out in a 2,300 m<sup>2</sup> plot cultivated with broccoli. This plot is located in the “Centro Integrado de Formación y Experiencias Agrarias (C.I.F.E.A.) in Lorca, province of Murcia (Spain). For a the two years study, the site was fertilized with different quantities of pig slurry. Samples of pig slurry and crops were analyzed for the most representative microorganisms (Coliforms, Aerobic Mesophile, moulds and yeasts, etc.) and also for the pathogens microorganisms *Salmonella*, *Shigella* and *Escherichia coli*.

All the slurry samples analysed contained high levels of total Coliforms (ranged from 10<sup>3</sup> to 10<sup>5</sup> coliforms/mL pig slurry), but most of the samples did not present faecals coliforms. Three of the eight samples analysed showed presence of *Salmonella*. *Shigella* was not found in any of the samples.

The analysis were carried out in plant-samples showed total coliforms, but at much lower levels than the samples of pig slurry (ranged from 10<sup>1</sup> to 10<sup>2</sup> coliforms/g of broccoli). However, total coliforms were observed, both in control plants (without pig slurry application) and in crops with pig slurry doses. Therefore, none of the samples contained food-borne pathogens *Salmonella*, *Shigella* and *E. coli*.

However, could not be found a direct correlation between coliforms and pathogens present in the pig slurry and in the crops.