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RESEARCH ARTICLE

On effect of poultry manure treatment with Effective Microorganisms with or without zeolite

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Abstract

The decomposition process of poultry manure is generally mediated by microorganisms, whose degradation activity has beneficial effects on soil fertility but, on the other hand, leads to the generation of malodour gas. Indeed, a relevant problem of poultry farms is represented by the release of bad smells, which are mainly a consequence of decomposition process of chicken feces, chicken bedding, plumes, dropped feed, and dust. Furthermore, the unpleasant odour, associated with poultry manure degradation, not only limits its use in agriculture but also negatively affects the housing communities located near the farms. This study aimed at evaluating the effects in vitro of different doses of Effective Microorganisms (EM), mainly consisting of live communities of lactic acid bacteria, photosynthetic bacteria, and yeasts, on poultry manure alone or with zeolite, a porous mineral with absorbent and ion-exchange properties, belonging to the family of aluminosilicates. The obtained results demonstrated that these treatments were able to reduce the poultry manure malodours, associated mainly with a decrease in the ammonia (NH_3) levels with respect to controls. The pH tended to increase, the nitrogen to go down, and the phosphorus to go up. Thus, all the effects described above were evident, testifying to a slower degradation of proteins, both with EM alone or in combination with zeolite. The presence of a pool of pesticides (65 components) was evaluated, and no variation was observed in the different experimental conditions versus control, as well as for REEs and metals. In conclusion, these preliminary results demonstrated that the use of EM with or without the addition of zeolite is a valid tool to eliminate the bad smell of manure and to make it a useful product as a fertilizer.

Keywords Ammonia (NH_3) · Effective Microorganisms · Environmental impacts · Poultry manure · Unpleasant odour · Zeolite

Introduction

Poultry production has increased over the past 30 years, rising from 91 million in 1990 to 365.85 million in 2021, due to the increased consumption of poultry meat and eggs (Livestock Economy at a Glance 2020–2021). As a result, the rapid growth of chicken farming worldwide has increased the supplies and requirements of the entire production chain. In particular, the poultry litter is one of the most critical points of this production chain, above all in the house flooring systems (Farghly et al. 2018), since it represents a waste of the production cycle (Hinkle 2010). The correct management of poultry litter is crucial for a safe poultry production chain, for human health protection, and for the odour pollution control (Ma et al. 2021). Diets administered to broilers and layer hens have relatively high protein content to guarantee high growth rates and to produce eggs of good quality. Consequently, the unused proteins are excreted in the form of uric acid containing nitrogen (N) in high concentration. In the environment, the excreted N (litter, feces, urine) is generally converted to ammonia (NH_3) by bacterial saprophytic microorganisms. NH_3 is the main atmospheric pollutant gas coming from poultry production, and it is of

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great concern above all for its impact on air quality, environment, and manure losses (Bist et al. 2023). Furthermore, it is recommended that NH_3 concentrations in the poultry farm should be never superior to 25 ppm, for its negative and dangerous effects also on human health and animal welfare (Bist et al. 2023). Besides ammonia (NH_3), poultry farms represent also one of the main emission sources of greenhouse gas (GHG), such as nitrous oxide (N_2O), carbon dioxide (CO_2), and methane (CH_4), which are known to be responsible for the current worrisome scenario of global warming contribute to global climate change (Anderson et al. 2021; Zisis et al. 2023). Therefore, poultry waste management constitutes a serious issue, which requires great attention, in order to reduce the impact of this challenging waste on the environment (Rahman et al. 2022). In literature, there are some reports describing the possibility to re-use poultry litter for other production chains only after being subjected to appropriate treatments, but often, it is not easy to put into practice in the farming reality (Bucher et al. 2020; Ma et al. 2021; Attia et al. 2022a; Ghanim et al. 2022).

In recent years, Effective Microorganisms (EM), consisting of live communities of beneficial microorganisms (predominantly lactic acid and photosynthetic bacteria, actinomycetes, yeasts, and fermenting fungi), have started to be considered as promising technology especially in agriculture where they have been employed as biofertilizer (Iriti et al. 2019). It has been reported that EM technology is an effective, promising, and sustainable tool that could be used not only to improve the soil health and properties, the germination potential and germination rate, but also to raise the yield and the quality of the products (Iriti et al. 2019). In addition, it has also been demonstrated that spraying rice seedlings with EM-activated liquid an increase in the leaf area, stem thickness, and chlorophyll content were obtained (Mosbæk et al. 1988; Jowett and McMaster 1995).

In poultry production, the use of EM could have good prospects firstly due to the benefits linked to their natural origin, but also to the possibility to improve the growth performances, to positively influence the gut microbiome composition, the intestinal histomorphology, and inevitably the animal health, to increase the carcass yield and quality of the obtained products (Awad et al. 2009; Ye et al. 2021; Yesuf et al. 2021). Indeed, the integration of EM into the drinking water and the spraying in the litter, but also the inclusion of EM in diets, has been shown to promote and improve the growth performance of broiler chickens (Mbaga and Mgunda 2013; Ye et al. 2021; Yesuf et al. 2021). What is more, it should be considered that the use of EM could be also beneficial to reduce the occurrence of bacterial infectious diseases (Weijiong and Yongzhen 2001) and, consequently, to limit the use of antibiotics that, for livestock production, were commonly used as antimicrobial growth promoters until they have been banned from the year 2006 (Regulation (EC) No 2821/98 and Regulation (EC) No. 1831/2003). In addition, the use of EM for a sustainable control of foodborne infections in food safety has been recognized (Gibby and Lancaster 2018).

In poultry facilities, however, odour control is one of the major problems to face and succeed in managing, above all when compared to confined livestock production systems (Ritter 1981). Some of the odour-causing compounds like ammonia are health hazards for people working or living near the farms but also for farmed animals (Mote 1984). Therefore, it would be strongly useful for poultry farmers to use poultry manure productively. The formation of ammonia, one of the main gases that generate the disgusting odours surrounding poultry facilities, has been attributed to the microbial decomposition of uric acid present in the manure (Carlile 1984). The rate of ammonia volatilization and resulting ammonia concentration in a chicken farm is usually related to several factors such as litter moisture content, pH, temperature, and wind speed (Moore et al. 1996). In this contest, the effect of EM in contrasting and reducing malodours could further be improved by using zeolites. Zeolites are three-dimensional, microporous crystalline minerals with well-defined structure of cavities and channels containing aluminium, silicon, and oxygen in their regular framework. For their characteristic structure, they are able to adsorb substances from the surrounding environment, allowing the control and the reduction of foul odours and air pollution during the biostabilization process (Omar et al. 2015).

The present study was conducted to evaluate the effect of EM, alone or together with zeolite, on poultry manure.

Materials and methods

Site of the trial and poultry farm

This experiment was carried out in summer 2021 in an open space with a sheet metal roof of a mechanical company located very close to a laying hens farm (certificated as *Salmonella* spp free), where poultry manure came from, in Nocera Inferiore (Salerno, Italy). The laying hens were housed on the ground with a litter surface of 250 cm^2/hen according to the EU regulations 1999/74/CE and 2002/4/CE. At the moment of litter sample collection, the hens were 55 weeks old and were fed by industrial feed mixtures (cereal grains, cereal by-products). After collection, the litter samples were moved to the site of the experiment in which environmental temperature and humidity were monitored.

Experimental materials

EM and zeolite were obtained from EM Schweiz, Regula Pedretti, In Cràna 2, 6724 Ponto Valentino/Valle di Blenio, Ticino (Switzerland).

Experimental design

The experimental design consisted of tests performed in triplicate with poultry litter, taken once in the poultry farm and distributed in plastic containers of 40 cm diameter and 28 cm high (2 kg of litter for each container) (Fig. 1). The containers were identified and divided into four groups: the control group (untreated); the EM group in which EM (diluted 1:10) was added and mixed to the litter at 100 ml/kg; the zeolite group in which zeolite was added and mixed to the litter at 300 g/kg; the EM + zeolite group in which EM and zeolite were added to the litter at 100 ml/kg and 300 g/kg, respectively.

Every day, the contents of the containers were turned at least twice (morning and afternoon) for 4 weeks. Every week, 60 g of sample was taken from each container to be used for chemical analyses.

Determination of antimicrobial residues

Eco Test Easy MRL (Calza Clemente s.r.l., Acquanegra Cremonese, Italy) was used for the determination of ß-lactams and tetracycline classes, which are frequently administered in veterinary practice. This test is generally validated for the detection of these antibiotics in raw or pasteurised cow, sheep or goat milk, or tank milk. In absence of specific commercial tests, for the detection of antibiotic residues in manure, this test kit was used to detect these antimicrobial residues in poultry manure previously diluted in sterilized distilled water (1/10). Positive and negative controls were included.

To confirm the obtained results, the same samples (10 µl of each sample, treated or untreated) were also tested by disk diffusion assay. Sterile blank disks of filter papers (Liofilchem, Teramo, Italy) were totally soaked into preprepared sample and placed on the surface of Mueller-Hinton agar plates (Liofilchem, Teramo, Italy) previously seeded with a bacterial culture. The used bacterial cultures were from the bacterial stocks stored at −80 °C in Microbank™ vials (Pro-lab Diagnostics, Richmond Hill, Canada) belonging to Microbiology Laboratory of the Department of Veterinary Medicine and Animal Production of the University of Naples "Federico II" (Naples, Italy). Two strains, a multidrug methicillin-resistant *Staphylococcus aureus* (*S. aureus*) strain and a strain of *S. aureus* susceptible to almost all antibiotics, both to a density of 0.5 McFarland, were selected for these assays. In addition to the disk diffusion test, a control blank disk was also added in every Mueller-Hinton agar plate. The plates were incubated at 37 °C for 24 h in aerobic conditions. The presence of antibiotic residues (positive results) was indicated by formation of an inhibition zone around the disk, as in Kirby-Bauer disk diffusion susceptibility test protocol.

Fig. 1 Site where the experiment was organized. **a** The phase of weighing the trays and adding manure. **b** Adding EM. **c** The mixing phase of the manure with a wooden big spoon

Chemical composition of the poultry manure

The pH was measured on around 30 g of fresh samples, in triplicate, using a pH-meter One Click™ (Mettler Toledo, Milan, Italy).

The NH_4^+-N in the poultry manure samples was determined with the phenol-hypochlorite colour development method, according to Solorzano (1969), using an ultraviolet-visible (UV-VIS) spectrophotometer. Samples were extracted using 2 M potassium chloride (KCl), and NH_4^+-N was analyzed using the indophenol blue method.

Total nitrogen (N) was determined using the Kjeldahl method, according to AOAC (2004) standard official procedures.

Samples were analyzed for total phosphorus (P) after a $HClO_4$–HNO_3 digestion according to Kuo (1996). Phosphorus concentration obtained from all extractions was measured using a spectrophotometer (Spectronic Genesys 8, Spectronic Instruments; Garforth, England) at 880 nm using the ascorbic acid method described by Kuo (1996).

Determination of metals and rare earth elements (REEs)

Metal analysis was carried out on the different samples of poultry manure treated and untreated in triplicate after acid digestion. About 0.5 g of the homogenised samples was subjected to microwave-assisted oxidative acid digestion using a mixture of hydrochloric acid, nitric acid, and hydrofluoric acid (6:3:1) at a temperature of up to 180 °C and high pressure for 40 min (Mars–CEM, Italy). Mineralized samples were recovered with ultrapure water up to a volume of 50 mL and analyzed by inductively coupled plasma–mass spectrometry (ICP-MS, Aurora M90 Bruker, Massachusetts, USA). The digestion step was performed using a blank sample to identify potential metal contamination of any of the materials and reagents used. A calibration curve was obtained for each analyzed element from a certified standard solution (Ultrascientific, Bologna, Italy). The detection limit (LOD) and limit of quantification (LOQ) were calculated using the range method of prediction to 95% of linear regressions, for each investigated metal. The calculated values of LOD and LOQ in the matrix 0.01 were 0.02 and 0.05 μg/kg, respectively.

Determination of pesticides

For pesticide determination, about 2.0 g of sample of poultry manure was extracted with 10 ml of an acetone-hexane mixture (1:1 v/v) using an ultrasonic extractor for 45 min (Brason, USA). The extract was concentrated by means of an evaporator under nitrogen flow to a final volume of 1 ml (Multivap10, Labtech, Italy). The extract was cleaned on a sulfate sodium anhydrous column, and then, 10 μL of the internal standard was added (mixture of 5 deuterated PAHs at the concentration of 10 mg L^{-1}) and injected to a gas chromatograph (Shimadzu 2010 Plus, Kyoto, Japan) coupled with a mass spectrometer (MS-TQ8030- Shimadzu, Kyoto, Japan) and a fused silica HP5-MS capillary column (30 m × 0.25 mm i.d.) with film thickness of 0.25 μm (Agilent Technologies, USA). The separation was conducted with oven temperature programmed as follows: initial setting at 80 °C (2 min hold) ramped to 180 °C at 20 °C min−1 and finally to 300 °C at 5 °C min (9 min hold). The injector was held at 280 °C. The mass spectrometer was operated in SIM (selected ion monitoring) mode. The quantification of pesticides was performed using an external calibration curve according to the internal standard method. The detection limit (LOD) and limit of quantification (LOQ) were calculated using the range method of prediction to 95% of linear regressions, for each investigated compound. The calculated average values of LOD and LOQ in the matrix of poultry manure were 0.007 μg/g and 0.02 μg/g. The data quality is ensured by certified reference, and the recovery percentage was estimated by analyzing in triplicate the CRM, and the range was 70–110%.

Statistical analysis

Data were analyzed by a two-way ANOVA model, using the GLM procedure of SAS (2000) and considering both the treatment and the week as main effects, as well as their interaction, according to the following model: $Yijk = m + Ti + Wj + T^*Wij + eijk$, where Y is the single observation; m is the general mean; T is the effect of the treatment: control, zeolite, EM, and EM + zeolite (i = control or ancient grains); W is the effect of the week (j = 1–5); and e is the error. The comparison of the means was performed by Tukey's test (SAS 2000). The results were expressed as average value, and the significance level was set at $P < 0.05$.

Results

Effects of tested treatments in poultry manure samples

The environmental temperature in the site of the experiment was ranged from 30 to 33 °C during the day and 27–28 °C at night. Relative humidity remained low (an average 69%) throughout the experimentation period. All 48 poultry manure samples, treated and untreated, were negative for the presence of antibiotic residues detected both by commercial kit and agar disk diffusion assay.

Table 1 reports the effect of zeolite and/or EM and time of treatment on chemical parameters of poultry manure. Zeolite

Table 1 Effect of the EM, with or without zeolite, on poultry manure treatment

	pH	NH_4^+-N ml/l	Ntot g/kg	Ptot g/kg
	Treatment effect			
Control	7.70B	16.14A	28.82B	12.76A
Zeolite	8.23A	13.84B	31.40A	11.08C
EM	8.07AB	15.87A	28.77B	11.84B
EM + zeolite	7.84AB	17.51A	29.49B	11.91B
	Week effect			
1	6.85B	20.18A	32.82A	10.95A
2	7.79AB	14.92BC	31.80A	11.52AB
3	8.44A	16.15B	29.32AB	12.33AB
4	8.44A	14.24C	27.72B	12.41AB
5	8.26A	13.68C	26.44B	12.27B
RMSE	0.42	2.03	0.766	0.608
	P values			
Treatment (T)	<0.0001	<0.0001	<0.0001	<0.0001
Week of trial (W)	0.0321	0.0170	0.0035	0.0431
T × W	0.2122	0.7369	0.0790	0.2576

EM Effective Microorganisms, *NH_4^+-N* ammonium, *Ntot* total nitrogen, *Ptot* total phosphorus. RMSE: within rows: A, B, and C: $P < 0.01$; *RMSE*, root mean square error

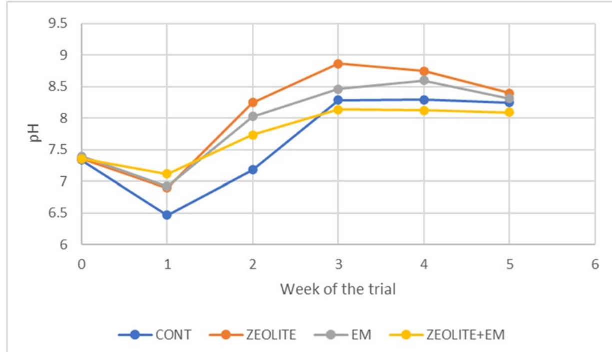

Fig. 2 Trend of pH of poultry litter during the weeks of the trial

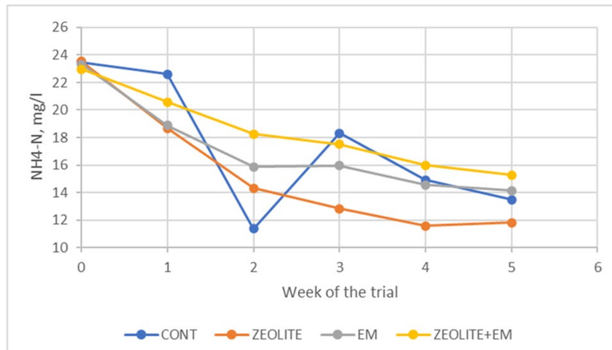

Fig. 3 Trend of ammonium (NH_4^+-N) in poultry litter during the weeks of the trial

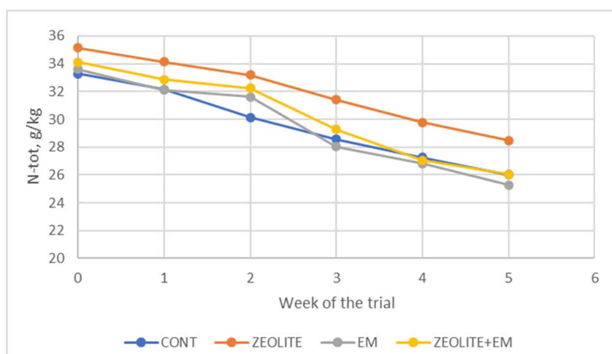

Fig. 4 Trend of total nitrogen (N) content in poultry litter during the weeks of the trial

alone was able to maintain the pH of the manure higher ($P < 0.01$) than the control, but this effect was not detectable when it was combined with EM. EM and zeolite+EM groups produced pH not different from that of the control group. Zeolite alone decreased the levels of NH_4 and increased the percentage of total nitrogen in poultry manure, while the other amendments were not able to affect these criteria compared to the control group. All the manure treatments were able to decrease the percentage of total P in the poultry manure, but the most effective was zeolite ($P < 0.01$). The weeks of treatment affected only total N and NH_4^+-N values, which decreased as the weeks of treatment increased, while pH and total *P* values tended to increase during the weeks of treatment. However, the effect of the interaction between the tested factor was not significant.

Figures 2, 3, 4, and 5 graphically represent the trend of the chemical constituents of poultry litter at the beginning of the trial (week 0) and during 5 weeks of the trial. In general, all the treated groups showed a similar trend, and only the trend of the control group was quite different (Figs. 2, 3, and 4). Regarding the *P*, there was a large variability among the treatment groups and the control group (Fig. 5).

Analysis of metals and rare earth elements (REEs)

The results of metals did not show bioaccumulation differences in the samples of poultry manure treated and untreated (Table 2). For the elements Be, B, Co, and Sn, small differences were observed which could be traced back to the different chemical composition of the samples. The concentration of metals (Al, Sb, As, Ba, Be, B, Cd, Co, Cr, Fe, Mn, Hg, Ni, Pb, Cu, Se, Sn, Tl, V, and Zn) and REEs was evaluated by ICP–MS analyses. The REE values did not show significant differences on all the samples analyzed (Table 3).

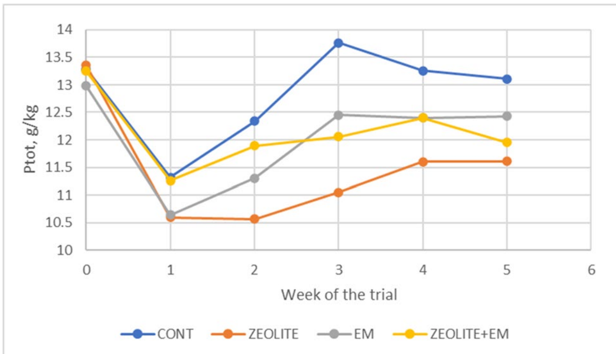

Fig. 5 Trend of the total phosphorus (P) in the poultry litter during the weeks of the trial

Analysis of pesticides

The analysis of about 60 pesticides showed the absence in all the samples examined (Table 4).

Discussion

Poultry litter is typically comprised of chicken excreta together with feathers, wasted feed, and a mixture of bedding materials, besides its own microbial communities (Hinkle 2010; Dumas et al. 2011). It is known that there are several factors influencing the poultry litter volatility, and among them, some are linked to litter such as pH and nitrogen content, others to the environmental conditions as temperature, humidity level, ventilation rate, and air velocity (Swelum et al. 2021). In poultry farms, ammonia (NH_3) is the main gas resulting from the chemical decomposition of uric acid operated by bacteria within the litter (Swelum et al. 2021). Generally, the bacterial degradation process begins when there are favorable environmental conditions and excreta on the litter, and it lasts until there is litter availability (Mendes et al. 2016). In poultry farms, the diets administered to chicken have a high content in proteins, to increase the growth performance and the carcass yield; consequently, the unmetabolized nitrogen is excreted in the feces, undergoing to a bacterial degradation process, releasing NH_4^+(Swelum et al. 2021). NH_4^+ undergoes conversion to NH_3, which being highly volatile, is dispersed into the air (Gates 2000). Indeed, NH_3, as a gas, is a dangerous threat not only for poultry and farm worker health but also for the environment contributing to air pollution (Ma et al. 2021; Swelum et al. 2021). Hence, different techniques have been utilized not only to contrast and reduce NH_3 emission and environmental dispersion, such as the amino acid supplementations to low dietary protein diets in broilers at different age stages or climate conditions (Attia et al. 2020; Attia

Table 2 Values of metals in treated and untreated poultry manure

	Al	Sb	As	Ba	Be	B	Cd	Co	Cr	Fe	Mn	Hg	Ni	Pb	Cu	Se	Sn	Tl	V	Zn
Control	2633	0.81	7.30	323	2.96	42.88	0.40	10.34	49.60	16,865	493	0.09	22.9	33.4	68.3	0.24	1.71	0.76	192	308
Zeolite	17,487	0.43	6.76	335	6.00	27.03	0.91	7.31	46.28	11,120	543	0.10	14.9	39.6	113	0.36	3.06	1.21	181	172
EM	2740	0.43	9.20	395	3.60	49.76	0.42	12.38	75.72	19,702	558	0.14	25.7	17.4	74.6	0.25	1.45	1.01	237	263
EM + zeolite	27,199	0.23	4.42	204	4.54	22.08	0.50	5.08	25.97	10,350	375	0.10	10.6	19.5	81.9	0.23	2.26	0.82	151	129
P values	0.277	0.114	0.056	0.287	0.003	0.018	0.110	0.014	0.081	0.058	0.050	0.056	0.065	0.382	0.249	0.825	< 0.001	0.047	0.053	0.051

The values of metals are mean the triplicates and expressed in milligram per kilogram

EM Effective Microorganisms, *Al* Aluminum, *Sb* Antimony, *As* Arsenic, *Ba* Barium, *B* Boron, *Cd* Cadmium, *Co* Cobalt, *Fe* Iron, *Mn* Manganese, *Hg* Mercury, *Ni* Nickel, *Pb* Lead, *Cu* Copper, *Se* Selenium, *Sn* Tin, *Tl* Thallium, *V* Vanadium, *Zn* Zinc

Table 3 REE values in treated and untreated poultry manure

	Sc	Y	La	Ce	Pr	Nd	Sm	Eu	Gd	Tb	Dy	Ho	Er	Tm	Yb	Lu
Control	55.8	13.2	28.2	60.8	6.0	20.7	4.4	1.26	4.4	0.67	2.3	0.38	1.3	0.15	1.1	0.19
Zeolite	28.5	16.2	29.0	62.4	5.7	18.0	3.6	0.74	4.1	0.62	2.6	0.46	1.6	0.22	1.5	0.26
EM	54.6	13.1	30.7	67.0	6.4	22.2	4.5	1.34	4.9	0.69	2.5	0.40	1.4	0.17	1.1	0.18
EM + zeolite	40.1	16.5	27.0	70.4	5.1	16.3	3.2	0.51	3.5	0.58	2.7	0.50	1.7	0.26	1.8	0.30
P values	0.319	0.243	0.710	0.672	0.471	0.139	0.148	0.120	0.272	0.433	0.479	0.050	0.096	0.067	0.073	0.064

The values of REEs are mean the triplicates and expressed in milligram per kilogram

EM Effective Microorganisms, *Sc* Scandium, *Y* Yttrium, *La* Lanthanum, *Ce* Cerium, *Pr* Praseodymium, *Nd* Neodymium, *Sm* Samarium, *Eu* Europium, *Gd* Gadolinium, *Tb* Terbium, *Dy* Dysprosium, *Ho* Holmium, *Er* Erbium, *Tm* Thulium, *Yb* Ytterbium, *Lu* Lutetium

Table 4 Value of pesticides in treated and untreated poultry manure

Compounds	Control	Zeolite	EM	EM + zeolite	Compounds	Control	Zeolite	EM	EM + zeolite
Chloroneb	< 0.02	< 0.02	< 0.02	< 0.02	2,4′-DDT	< 0.02	< 0.02	< 0.02	< 0.02
Pentachlorobenzene	< 0.02	< 0.02	< 0.02	< 0.02	cis-Nonachlor	< 0.02	< 0.02	< 0.02	< 0.02
BHC, alpha	< 0.02	< 0.02	< 0.02	< 0.02	Endrin aldehyde	< 0.02	< 0.02	< 0.02	< 0.02
Hexachlorobenzene	< 0.02	< 0.02	< 0.02	< 0.02	2,4′-Methoxychlor	< 0.02	< 0.02	< 0.02	< 0.02
Pentachloroanisole	< 0.02	< 0.02	< 0.02	< 0.02	Endrin ketone	< 0.02	< 0.02	< 0.02	< 0.02
BHC, beta	< 0.02	< 0.02	< 0.02	< 0.02	Tetradifon	< 0.02	< 0.02	< 0.02	< 0.02
BHC, gamma	< 0.02	< 0.02	< 0.02	< 0.02	Mirex	< 0.02	< 0.02	< 0.02	< 0.02
BHC, delta	< 0.02	< 0.02	< 0.02	< 0.02	Dichlorvos	< 0.02	< 0.02	< 0.02	< 0.02
Endosulfan ether	< 0.02	< 0.02	< 0.02	< 0.02	Diclobenil	< 0.02	< 0.02	< 0.02	< 0.02
Heptachlor	< 0.02	< 0.02	< 0.02	< 0.02	Atrazina desetil	< 0.02	< 0.02	< 0.02	< 0.02
Pentachlorothioanisole	< 0.02	< 0.02	< 0.02	< 0.02	Trifluralin	< 0.02	< 0.02	< 0.02	< 0.02
Aldrin	< 0.02	< 0.02	< 0.02	< 0.02	Simazina	< 0.02	< 0.02	< 0.02	< 0.02
4,4′-Dichlorobenzophenone	< 0.02	< 0.02	< 0.02	< 0.02	Atrazina	< 0.02	< 0.02	< 0.02	< 0.02
Fenson	< 0.02	< 0.02	< 0.02	< 0.02	Terbumenton	< 0.02	< 0.02	< 0.02	< 0.02
Isodrin	< 0.02	< 0.02	< 0.02	< 0.02	Fonofos	< 0.02	< 0.02	< 0.02	< 0.02
Heptachlor epoxide	< 0.02	< 0.02	< 0.02	< 0.02	Propizamide	< 0.02	< 0.02	< 0.02	< 0.02
Chlorbenside	< 0.02	< 0.02	< 0.02	< 0.02	Terbutilazina	< 0.02	< 0.02	< 0.02	< 0.02
trans-Chlordane	< 0.02	< 0.02	< 0.02	< 0.02	Terbufos	< 0.02	< 0.02	< 0.02	< 0.02
2,4′-DDE	< 0.02	< 0.02	< 0.02	< 0.02	Tolclofos metile	< 0.02	< 0.02	< 0.02	< 0.02
Endosulfan I	< 0.02	< 0.02	< 0.02	< 0.02	Alachlor	< 0.02	< 0.02	< 0.02	< 0.02
cis-Chlordane	< 0.02	< 0.02	< 0.02	< 0.02	Metalaxil	< 0.02	< 0.02	< 0.02	< 0.02
trans-Nonachlor	< 0.02	< 0.02	< 0.02	< 0.02	Fenclorfos	< 0.02	< 0.02	< 0.02	< 0.02
Chlorfenson (Ovex)	< 0.02	< 0.02	< 0.02	< 0.02	Malation	< 0.02	< 0.02	< 0.02	< 0.02
4,4′ DDE	< 0.02	< 0.02	< 0.02	< 0.02	Metolaclor	< 0.02	< 0.02	< 0.02	< 0.02
Dieldrin	< 0.02	< 0.02	< 0.02	< 0.02	Fention	< 0.02	< 0.02	< 0.02	< 0.02
2,4′-DDD	< 0.02	< 0.02	< 0.02	< 0.02	Bromofos metil	< 0.02	< 0.02	< 0.02	< 0.02
Endrin	< 0.02	< 0.02	< 0.02	< 0.02	Clorfenvifos	< 0.02	< 0.02	< 0.02	< 0.02
cis-Nonachlor	< 0.02	< 0.02	< 0.02	< 0.02	Procimidone	< 0.02	< 0.02	< 0.02	< 0.02
Endosulfan II	< 0.02	< 0.02	< 0.02	< 0.02	Bromofos etil	< 0.02	< 0.02	< 0.02	< 0.02
4,4′-DDD	< 0.02	< 0.02	< 0.02	< 0.02	Tetraclorvinfos	< 0.02	< 0.02	< 0.02	< 0.02
2,4′-DDT	< 0.02	< 0.02	< 0.02	< 0.02	Oxadixil	< 0.02	< 0.02	< 0.02	< 0.02
2,4′-Methoxychlor	< 0.02	< 0.02	< 0.02	< 0.02	Endosulfan sulfate	< 0.02	< 0.02	< 0.02	< 0.02
Endrin ketone	< 0.02	< 0.02	< 0.02	< 0.02	4,4′-DDT	< 0.02	< 0.02	< 0.02	< 0.02

The value of pesticides is the mean of the triplicates and expressed in milligram per kilogram

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et al. 2022b), but also to suppress manure malodours, for example, the addition to litter of amendments as aluminun sulfate/sodium bisulfate, enzyme inhibitors, and gas absorbers (Swelum et al. 2021; Attia et al. 2022a), as done by us in this trial testing the use of zeolite and EM on poultry manure. Several studies reported the ability of EM to suppress manure malodours in poultry farms (Ma et al. 2021), besides to improve the growth performance of broilers and strengthen their immune system by adding EM in feed and drinking water (Awad et al. 2009; Ye et al. 2021; Yesuf et al. 2021). Indeed, EM contains beneficial microorganisms which can colonize the animal gut when administered with feed and drinking water, ameliorating gut histomorphology and physiological properties (Awad et al. 2009). Moreover, EM are able to contrast the proliferation and the degradative activity of the indigenous putrefactive bacteria, which are generally responsible for the bad smell of manure and for the transformation of proteins and amino acids into NH_3-N and NH_4^+-N, as reported by Weijiong and Yongzhen (2001). Weijiong and Yongzhen (2001) demonstrated that the addition of EM to drinking water and feed allowed a reduction of poultry manure malodours in association with a relevant decrease of NH_3 levels (i.e., 42 to 70% lower in treated trials than the controls). Furthermore, they proved that EM were able to increase of 28% the amino acid content of the EM inoculated and fermented feed (Weijiong and Yongzhen 2001).

In this study, the efficacy of EM or zeolite (100 ml/kg and 300 g/kg, respectively), alone or together, on poultry manure was compared. Zeolites exhibit high ability to adsorb ammonium and nitrate ions, inhibiting the conversion to free ammonia. Thereby, zeolites can control nitrogen losses and reduce odours and air pollution during the biostabilization process, while the temperature influences the microbial activity during composting (Omar et al. 2015). Our experimental study was carried out in the height of summer with an average temperature of 28 °C. Among the different tested treatments, the zeolite was the most effective in modifying the chemical characteristics of poultry manure, significantly affecting all the tested criteria. The litter pH is an important element in regulating the NH_3 volatilization. It specifies the ratio of volatile NH_3/ammonium (NH_4^+), the ionic and non-volatile forms of NH_4^+–N. NH_3 accumulation in poultry houses mainly depends on the bird's weight and ventilation rate (Swelum et al. 2021). Zeolite was the only treatment able to significantly modify the pH in comparison with the control group. The changes in manure pH due to the addition of zeolite were similar to that recorded by Singh et al. (2016), Huang et al. (2017), and Šubová et al. (2021). In general, the pH in the manure tends to decrease with time up to 3–4 weeks of composting, due to the nitrification process producing H+ (Lim et al. 2017). The effect of zeolite on manure pH could be explained to its absorption and cation exchange ability. Effective Microorganisms (EM) induced pH values similar to zeolite group but also not different than the control one. Interestingly, when zeolite was combined with EM, the positive effects on the pH could not be detected, and these interesting obtained results need further investigations. To the best of our knowledge, there are no other studies in the literature analyzing the combined activity of zeolite and EM. Consequently, a possible hypothesis is that in this group, there was an interference between zeolite and EM. In fact, the negative charge of alumino-silicate framework could be naturally counter-balanced by alkaline or alkaline-earth metal cations such as sodium (Na+) and potassium (K+). However, it is possible also the combination with other cations such as heavy metals (Rožić et al. 2000). As a result, the zeolites can acquire biocide activities against Gram-positive and Gram-negative bacteria, as reviewed by Torres-Giner et al. (2017), and N losses during composting are associated mainly with the following three mechanisms: (a) volatilization of NH_3 at high temperatures and high pH values; (b) NOx volatilization attributed to nitrification and denitrification; (c) loss of water-soluble nitrogen due to leachate (Swelum et al. 2021). The addition of zeolite (300 g/kg) to poultry litter samples significantly reduced the release of NH_4-H^+ in comparison to the other treatments, and, as a consequence, the amount of total nitrogen was higher. This obtained result agrees with what reported by Šubová et al. (2021). None of the other treatments was able to affect the nitrogen metabolism in poultry litter.

Regarding the total phosphorus (P) content of the poultry litter, all the treatments decreased its value, but zeolite was again the most effective one. The reduction of P percentage was not particularly high, but it was mainly due to the form of P in poultry manure. During composting, P is lost by the leachate in the form of HPO_4^{2-}, H_2PO_4. Compared to other animal manures, poultry manure contains proportionally more of the stable form of P, which ranges from 22 to 58% of total phosphorus (Dail et al. 2007). It is the phytic phosphorus of which the diets for poultry are particularly rich, due to the inclusion of a large amount of cereal grains, mainly represented by corn. As the phytic P is undigestible for poultry, farmers normally supplement the diets with organic source of P, and, for this reason, poultry manures are, in general, richer of P in comparison to manure from other species (Kacprzak et al. 2022). However, all the treatments tested in this trial were able to give a better stability of poultry litter in terms of pH, NH_4^+, and Ntot. The large variability of the effects on P among the treatments could be ascribed to the nature of the P in the poultry litter (phytic and organic) that could affect the activity of the microorganisms.

Conclusions

These experimental results indicated that EM and zeolite use in poultry litter management has great potential for suppressing malodours of manure. Indeed, EM are often used as fertilizer, due to their ability not only to degrade organic residues but also to control the emission of odours and to transform nutrients in forms assimilable by plants, while zeolite, due to its chemical characteristics, has a great potential for use in poultry farming for its good activity in controlling NH_3 losses and consequently in reduction of environmental pollution. Therefore, the inoculation of EM and zeolite in poultry manure represents a reasonable strategy to improve sustainable production and to protect the environment, all on a cost-effective basis.

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Data Availability Not applicable

Declarations

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Consent to participate Not applicable

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