The Asian and Australasian Journal of Plant Science and Biotechnology ©2012 Global Science Books

Comparing the Effect of Extractor Types and Substrate on Vermiwash Quality

Samaneh Rahmatpour* • Hossen Ali Alikhani • Leila Mohammadi

Departament of Agriculture and Natural Resources, University of Tehran, 31587 Iran

Corresponding author: * samanehrahmatpour@gmail.com

ABSTRACT

Few reports are available on the effect of extractor characteristics and substrate on vermiwash quality. Therefore, the aim of the present study was to determine the effect of different kinds of extractor and substrate on the quality of vermiwash. Completely randomized design was performed in a factorial arrangement with 15 treatments (3×5, three substrate and five kinds of extractor), each in 4 replicates. Salinity, respiratory activity, some of macro and micro nutrient and microbial population were measured in vermicompost tea produced. The results showed that using DTPA extractor increased the amount of all minerals compound except potassium in vermiwash significantly ($P < 0.05$). The highest level of Nitrogen concentration was in the extracted vermiwash by distilled water with pH=5. Extraction by distilled water with pH=9 and DTPA solution extractors showed the maximum level of total Iron in this study. Total microbial population did not differ with extraction type, but maximum respiration rate was observed in the extracted vermiwash by DTPA solution extractor. Maximum elements concentration was observed in the extracted vermiwash of immature vermicompost, excluding potassium and nitrogen in maximum concentration that existed in the extracted vermiwash of earthworms. Generally, distilled water solutions were comparable with DTPA solution and the extracted vermiwash of immature vermicompost was the best substrate. Thus, the results clearly suggested that preparation methods of vermiwash may influence the properties of final vermiwash. The increased level of plant nutrients in final products in different methods demonstrated that the vermiwash could be a valuable biofertilizer for sustainable farming practices.

Keywords: DTPA, earthworm, pH, vermicompost

Abbreviations: CRD, completely randomized design; **CW**, composter worm; **DTPA**, diethylene triamin penta acetic acid; **EDTA**, ethylene diamine tetra acetic acid; **HSD**, honestly significant difference; **IV**, immature vermicompost; **LSD**, least significant difference; **MPN**, most probable number; **MV**, mature vermicompost; **TEA**, three ethanol amine; **TP**, total phosphorus; **VC**, vermicompost

INTRODUCTION

As the global food demand is expected to be doubled in the next 50 years, it poses huge challenges for the sustainability both of food production and of terrestrial and aquatic ecosystems and the services they provide to society. Much attention has been paid in recent years to manage different organic wastes resources at low input as well eco-friendly basis. Animal manure application to soils in arid and semi-arid areas was a traditional source of nutrients and organic matter for soil-plant systems. These wastes seldom can be applied directly in the soil because they damage soil fertility and lead to structural incompatibility, the immobility of nitrogen and phytotoxicity. Some kind of treatment on vermicompost (VC) and vermiwash can make these wastes suitable for application in soil and safe repulsion in environment.

Until recently, compost tea has been defined simply as a liquid extract from composted material that may contain organic and inorganic soluble nutrients, and a large number of organisms including bacteria, fungi, protozoa and nematodes (Recycled Organics Unite 2003). Vermiwash contains an enzyme cocktail of proteases, amylases, urease and phosphatase. Microbiological study of vermiwash revealed that it contains nitrogen-fixing bacteria like *Azotobactrer* sp., *Agrobacterium* sp. and *Rhizobium* sp. and some phosphate-solublizing bacteria. Vermiwash is a collection of excretory products and excess secretions of earthworms along with micronutrients from soil organic molecules.

Teas from VC could possess some of the same beneficial microbiological and chemical characteristics of solid VCs. During "brewing" process, soluble mineral nutrients, beneficial microorganisms, humic and fulvic acids, plant growth hormones and plant growth regulators-known to be available in solid VC-are probably extracted into the tea. Composting feedstock influence the biological, chemical and physical character of finished compost, which, in turn impacts the characteristics and efficacy of the derived compost tea. The advantage of animal manure compost is the diversity in microbial populations (Scheuerell 2003), or the higher levels of available phosphorus, calcium and trace elements, as compared to yard and lawn trimmings (Pittway 2003). The selection of compost characteristics depends upon the type of compost tea that required and the purpose of its use (Ingham 2005). A highly aerobic compost made from a mix of manure and plant material, is favored (Grobe 2003). Aerobic compost is considered to maintain a habitat that allows beneficial organisms to dominate and compete with less beneficial and non-beneficial organisms that tend to populate more rapidly in the reduced oxygen conditions (Ingham 2005). Based on a two-year field experiment, Welke (2005) concluded that both aerated and non-aerated compost teas extracted from composted animal manure for 7 days had positive and similar effects on strawberry yield and suppression of *Botrytis cinerea*. In contrast, Arancon *et al*. (2007) reported that aerated vermicompost tea had a more positive impact on plant growth than non-aerated tea extracted for the same period of time (24 h).

According to Arancon *et al*. (2007), the ratio of solids to water used can range from 1:3 (33%) to 1.200 to (0.5%). Water-extractable growth regulators or phytohormones extracted from vermicompost may also have a positive effect

Received: 29 December, 2011. Accepted: 3 December, 2012.

Original Research Paper

on initial root development and plant growth (Keeling 2003; Edwards *et al*. 2006).

DTPA is a chelating agent because it can effectively extract micronutrient such as Fe, Zn, Cu and Mn. Some Factors including pH, chelating agents' concentration, time of shaking and temperature can influence the extracted micronutrient. Usually these factors were adjusted for producing optimum vermiwash (Lindsy and Norvell 1978). The study conducted by Rosa *et al*. (2002) is known as "Extraction and exchange behavior of metal species therapeutically applied peat". The binding and availability of metals in therapeutically applied peat were characterized by means of a versatile extraction approach. Aqueous extracts of peat were obtained by using high-purity water (pH 4.5 and 5.0), $CaCl_2$, EDTA and DTPA solution as metal extractors. The efficiency of the applied extractors follows this order: DTPA=EDTA solution >> $CaCl_2$ solution > water of pH 5.0>water of pH 4.5. The following order of metal availability on the EDTA and DTPA solution could be found: Ca, Mg, Mn >> Zn > Pb > Cd > Ni > Co > Fe > Sr, Al. Also, this study shows the pH value can exert a strong influence on the remobilization of metals. Even at a slightly raised pH value, however, metals are increasingly remobilized, particularly Al and Fe. Concentrations of Ca, Mg extractable at pH 4.5 and 5.0 indicate that these metals are rather weakly bound to peat matter and can be remobilized even by water. Therefore the aim of the present study was to determine the effect of different kinds of extractor and substrate on the quality of vermiwash.

MATERIALS AND METHODS

Preparation of aerobic vermiwash

In these experiments, earthworms *Eisenia foetida* and VC were provided from VC Research Center of Faculty of Agricultural Engineering and Technology in Tehran University.

VCs substrate was cow manure and Sycamore leaf with 2:1 ratio. Immature VC was collected on 60th day and the mature VC on 90th day.

At first, mature and immature VC were air dried, passed through a two mm sieve and extracted. VC and extractor were mixed in the ratio of 1:5 (w/v), and the ratio of earthworm to extractor was 1:2. The experiment was fulfilled in a factorial completely randomized design (RCD) with four replicates. Vermiwash was prepared using three types of substrate, namely, 1 - mature VC (MV) 2 - immature VC (IV) 3 - composter worm (CW). Aqueous extracts of VC and earthworm were obtained using high-purity water (pH 9, 5.0 and 7.0), DTPA full strength and DTPA 1/3 strength. 0.005 M DTPA with 0.1M Three Ethanol Amine (TEA) and 0.01 M CaCl2 dissolve in a liter distilled water. pH value of DTPA solution was adjusted to 7.3 (Lindsy and Norvell 1978). The mixtures were supplied with aeration using a shaker for 30 min (150 rpm) and filtered through S&S 598.2 filter paper.

Chemical analysis

The chemical analysis of VC and vermiwash was conducted using different methods. Electrical Conductivity was measured by an EC-meter Jenway-4510 and pH by pH-meter PHM-2000. Total Kjeldahl Nitrogen was determined by Sparks (1996) procedure. Total Phosphorus (TP) by sulfate acid digestion (Hosomi and Sudo 1986) was followed by vanadomolybdate method for P analysis (Ryan *et al*. 2005); Total Magnesium and Calcium and total Zn and Fe by nitric acid digestion (American Public Health Association, 1998) were also followed by the atomic absorption spectrometry (A-670) for Fe an Zn analysis and complexometeric for Ca and Mg analysis potassium by flame photometer (ELE). The Most Probable Number (MPN) in vermiwash was determined by serial dilution method (Alef and Nannipieri 1995) and respiration was measured too (Page *et al*. 1982). Coliforms population was measured by producing gas in Durham tubes, including Lactose Broth media, and incubated for 48 h at 35°C (Alef and Nannipieri 1995). The chemical analysis methods of VC are described in the following table (**Table 1**).

Table 1 Analytical methods of vermicompost.

Garg *et al*. 2006	EC and pH
Nelson and Somers 1975	OC
Bremmer and Mulvaney 1982	N
Olsen and Nelson 1982	P
Jackson 1958	Ca+ Mg
Jackson 1958	K
Olsen and Roscoe 1982	Fe
Backer and Amacher 1982	Zn
Alef and Nannipieri 1995	MPN

Statistics

Data were subjected to analysis of variance and tested for significance by Turkey's test (HSD) and Least Significant Difference (LSD) using SPSS 13.0 and MSTAT.EXE. All the experiments were carried out twice and data was pooled before statistical analyses.

RESULTS AND DISCUSSION

The chemical characteristics and microbial population of MV and IM are observed in **Table 2**. The electrical conductivity in MV and IM was 2.5 dsm^{-1}. MV had a lower pH (**Table 2**), which may be due to the accumulation of organic acids from microbial metabolism or from the production of fulvic and humic acids during decomposition (Venkatesh and Eevera 2008). The C: N ratio, as one of the most widely used indices for maturity of organic wastes, decreased with time due to decomposition (**Table 2**). Initial C: N ratio was 25.314. Final C: N ratio was 33.982. Suthar (2008) reported that the C: N ratio of substrate material reflects the organic waste mineralization and stabilization during the process of decomposition. The loss of carbon as CO_2 through microbial respiration added nitrogenous excretory material between the C: N ratio of the substrate. C: N ratio is one of the most widely used indicators of VC maturation which decreases sharply during vermic process (Kale 1998; Gupta and Garg 2007; Suther 2008; Venkatesh and Evera 2008). Elvira *et al*. (1998) stated that a large fraction of organic matter in the initial substrate was the loss of CO_2 between 20 and 43% which was as total organic carbon by the end of vermicomposting.

Elvira *et al*. (1998) and Suthar (2007) reported that body fluid and excreta secreted by earthworm (e.g. mucus, high concentration of organic matter, ammonium and urea) promoted microbial growth vermicomposting.

Table 2 Analysis of mature and immature vermicompost (VC).

	Mature VC	Immature VC
pH	7.1	7.4
EC (dsm^{-1})	2.43	2.53
OC%	54.45	62.37
Total N (mg/kg)	2.151	1.891
Total P %	0.337	0.404
C/N	32.982	25.314
Total K (meq/L)	70.97	212.78
Total Ca + Mg (meq/L)	255	280.5
Total Fe (mg/kg)	2035.8	2361.8
Total Zn (mg/kg)	1434	1882
MPN	$2.1*10^8$	$2.7*10^{14}$

Table 3 Effect of Substrate on vermiwash EC.

Substrate	EC (ds m^{-1})
MV	2.553 b
IV	3.938 a
CW	2.535 b

MV, mature vermicompost; IV, immature vermicompost; CW, composter worm.
*Different letters within a column indicate significant differences according to Duncan's multiple range test ($P < 0.05$)

Table 4 Nutrient content in vermiwash obtained from different substrate.

substrate	Ca [b]	Mg [b]	K [b]	P [a]	N [a]	Zn [a]	Fe [a]
Mature VC	16.82 b	5.067 b	114.6 c	1.311 c	17.15 b	1.572 a	1.654 b
Immature VC	18.52 a	6.897 a	141.1 b	2.253 a	16.10 c	1.797 a	2.179 a
Earthworm	12.52 c	2.983 c	461.3 a	1.984 b	19.80 a	1.294 a	2.717 a

[a] mg L^{-1}
[b] meq L^{-1}
VC: vermicompost
* Different letters within a column indicate significant differences according to Duncan's multiple range test ($P < 0.05$).

The effect of substrate

Results of the analysis of variance indicated that there were significant differences ($P < 0.05$) on EC (**Table 3**). The EC was decreased from immature VC to mature VC (3.94 – 2.53), showing the decrease in EC during vermicomposting.

The volatilization of ammonia and the precipitation of mineral salts could be the possible reasons for the decrease in EC at vermicomposting (Venkatesh and Eevera 2008). Also, Nath *et al.* (2009) reported that the EC was reduced in the range of 30 to 48% for different feeds after vermicomposting. The increase in EC might have been due to the loss of weight of organic matter and release of different mineral salts in available forms (Wong *et al.* 1997; Kaviraj and Sharma 2003).

Substrate type showed significant differences ($P < 0.01$) on total calcium, magnesium and phosphorus concentration. Most total calcium (18.52 meq/L) and magnesium (6.897 meq/L) concentration was observed in the vermiwash producing IV. Total calcium and magnesium concentration was reduced by 10 and 36%, respectively in the MV-produced vermiwash (**Table 3**). According to Venkatesh and Eevera (2008), total calcium remained constant after the 60th day of vermicomposting.

Partasarathi and Ranganatan (1998) observed that aged casts showed reduced enzyme activities because of decreased moisture content, lower nutrient concentrations and a decline in microbial activity. Nath *et al.* (2009) reported total calcium concentration was higher in final products than the initial feed mixture (**Table 4**). Elvira *et al.* (1996) suggested that gut processes associated with calcium metabolism are primarily responsible for enhanced content of inorganic calcium content worm cast.

Results of the analysis of variance indicated that substrates had significant differences ($P < 0/05$) on total potassium concentration (**Table 4**). The highest amount of total potassium (461.3 meq/L) was recorded in the produced vermiwash of earthworm that probably correlated with digestion system earthworm.

Results of the analysis of variance indicated that substrates had significant differences ($P < 0.05$) on total nitrogen (**Table 4**).

Produced vermiwash of CW and IV, respectively had the most (19.80 mg/L) and the least (16.10 mg/L) total nitrogen content, respectively. The increase of in the nitrogen value (6.52%) during vermicomposting process is as a result of carbon loss and probably because of mineralization of organic matter (Venkatesh and Eevera 2008). Final nitrogen content is dependent on the initial N present in the waste and the extent of the decomposition (Crawford 1983). Total nitrogen content was increased in final products of different vermin beds because of organic matter mineralization (Nath *et al.* 2009). Losses of organic carbon might be responsible for nitrogen addition in the form of mucus nitrogenous excretory substances, growth stimulatory hormones and enzymes from the gut of earthworms (Tripathi and Bhardwaj 2004). Sharma *et al.* (2011) reported that the increase in shoot extension growth might be due to increased uptake of nutrients and increased release of growth factors in the root zone. The application of bioorganic resulted in increased N uptake and release of growth-promoting substances such as auxins, gibberellins, and cytokinins in the root zone. Further, *A. chroococcum* has also been reported to improve biological nitrogen fixation (BNF) and its quick release for plants absorption.

Table 5 Effect of substrate on vermiwash MPN.

Substrate	**MPN** (NUml^{-1})
MV	6.722 a
IV	6.888 a
CW	7.271 a

MV, mature vermicompost; IV, immature vermicompost; CW, composter worm.
*Different letters within a column indicate significant differences according to Duncan's multiple range test ($P < 0.05$)

Table 6 Effect of substrate on vermiwash respiration.

Substrate	Respiration (mg CO_2/ml.day)
MV	0.9724 b
IV	1.081 a
CW	0.792 c

MV, mature vermicompost; IV, immature vermicompost; CW, composter worm.
*Different letters within a column indicate significant differences according to Duncan's multiple range test ($P < 0.05$)

Significant increases were observed in teas obtained from earthworm and immature VC (**Table 4**). The earthworm body wash indicates the presence of some plant growth promoters which caused a significant effect on plant growth as well as the biochemistry of seedlings. Suther (2010) indicate that vermiwash may be utilized effectively for sustainable plant production at low input-basis green framings. Suther (2011) observed that the increase in the EC, N, P, K and micronutrients (Cu, Zn, Mg and Fe) in all worm worked composts (except in the case of N in neem leaves compost) showed that the activity of earthworm *E. eugienae* along with microorganisms promoted mineralization process and brought the nutrients to ready use for plant growth (Suther 2011).

The results indicated that the total iron content was decreased in the vermiwash produced from MV (31.74%). Partasarathi and Ranganatan (1998) reported a decrease in enzyme activities and nutrient content in worm casts by passing the time.

Type of substrate didn't have any significant effect on microbial population (**Table 5**), but an increasing in MPN was observed in the vermiwash produced from CW against MV and IV. Type of substrate had a significant effect on respiration activity (**Table 6**). vermiwash produced from IV had the most respiration activity (1.081 mg CO_2/ml.day). Many studies have shown an increase in some reproductive microorganisms on the earthworm's gut (Byzov 2007; Parthasarathi *et al.* 2008). Studies suggested that microbes associated with earthworm body probably produce auxin and gibberellins-like substances, which can promote seedling, germinates and growth in plants. Moreover, there is evidence that earthworms alter plant seed germination and seedling establishment indirectly via excreta (Eisenhauer *et al.* 2009). The Partasarathi and Ranganatan (1998) study revealed that the reduced enzyme activities in aged casts of both *L. mauritii* and *E. eugeniae* were probably due to: (1) their reduced moisture and nutrient contents, (2) a decline in or inactivation of the microbial population and, (3) low stability of aged earthworm casts.

Although respiratory CO_2 release is linearly proportional to substrate availability, the rate at which the substrates are converted to CO_2 varies with substrate types (Berg *et al.* 1982). Simple sugars can be readily converted to CO_2 by roots and microbes with short residence times. It can be very difficult for humic acids to be decomposed and converted to CO_2 with residence times of hundreds or thousands of years.

Table 7 Effect of extractor on EC and Ca, Mg, K and N content in vermiwash.

Extractor	K	Mg	Ca	EC	N
Distilled water with pH=5	302.1 a	3.189 c	9.139 c	1.704 cd	18.67 ab
Distilled water with pH=7	259.4 b	3.083 c	10.58 c	1.611 d	17.50 b
Distilled water with pH=7	222.2 bc	4.444 b	8.583 c	1.771 c	16.92 b
1/3DTPA solution	204.20 c	4.556 b	19.61 b	3.436 b	18.33 ab
DTPA solution	207.30 c	9.639 a	31.83 a	6.521 a	19.00 a

[a] mg L^{-1}
[b] dSm^{-1}
*Different letters within a column indicate significant differences according to Duncan's multiple range test ($P < 0.05$).

Table 8 Effect of extractor on P, Fe, Zn content and MPN and respiration activity in vermiwash.

Extractor	Log_{10} MPN [a]	Zn [b]	Fe [b]	P [b]	Respiration [c]
Distilled water with pH=5	7.243 a	0.9467 c	1.646 c	1.649 b	0.1522 c
Distilled water with pH=7	6.703 a	1.214 c	1.999 bc	1.636 b	0.1247 c
Distilled water with pH=7	7.308 a	1.395 bc	2.790 a	1.604 b	0.1870 c
1/3DTPA solution	6.999 a	2.283 a	2.028 b	2.265 a	1.5330 b
DTPA solution	6.549 a	1.934 ab	2.455 ab	2.092 a	2.746 a

[a] $NUml^{-1}$
[b] mgL^{-1}
[c] $mgCO_2$/ml. day
*Different letters within a column indicate significant differences according to Duncan's multiple range test ($P < 0.05$).

We observed that the coliform population was reduced by 100% in MV as compared with IV. It suggests that digestive processes in the gut of *E. fetida* are the main factors involved in the decrease of total coliforms observed in the mature VC. Decreases in total coliform numbers were not related to decreases in bacterial biomass, indicating a specific negative effect of earthworms on the coliforms (Monry *et al*. 2009). The vermicompost leachate was free of pathogens, i.e. coliforms (*E. coli*), *Salmonella* sp. and *Shigella* spp. The reduction of pathogens in the vermicompost might be related to the release of coelomic fluids by the earthworms during vermicomposting, which could have antibacterial properties and kill pathogens (Valembois *et al*. 1982; Gutiérrez-Miceli 2008).

Type of extractor

The effect of extractor was significant ($P < 0.01$) on EC in vermiwash (**Table 7**). Higher EC observed in the extracted vermiwash by DTPA (6.21 dSm^{-1}) and 1/3 DTPA (3.436 dSm^{-1}) solutions was probably the reason for high strength chelating element by DTPA of substrate.

So it had been observed that each extractor extracts different quantities of elements from substrate. Type of extractor had a significant effect on total calcium, magnesium and potassium content in vermiwash ($P < 0.01$). The highest amount of total calcium (31.83 meq/L), magnesium (9.639 meq/L) and phosphorus (2.265 mg/L) concentration existed in the extracted vermiwash from DTPA solution (**Table 7**). Probably, it is because of high strength chelating cations by DTPA that it is the same as that Rosa *et al*. (2002) report. Gholizadeh (2000) reported more element derivation by agents chelating. This is because the decrease in agents chelating free metal ions in solution via producing metal-chelate soluble complexes and for recovery of this reduction, extracted ions from solid phase (exchange, sediment on carbonates, complexes with organic material and amorphous sesquoxids) are needed.

Extractor had a significant effect on total potassium of vermiwash (**Table 7**). Results showed that using distilled water with pH=5 extractor increased the potassium content (302.1 meq/L) in vermiwash significantly ($P < 0.05$). As far as the charge of organic material was partly pH-dependent, the charge of this part was reduces by pH decline, causing an increase in soluble Potassium (Ardalan and savaghebi 2001; Salardini 2002).

Extractor was significantly different regarding nitrogen content in vermiwash (**Table 7**). The extracted vermiwash by DTPA had the highest nitrogen content (19 mg/L) in comparison with other treatments.

Type of extractor had also a significant effect on iron content in vermiwash ($P < 0.05$). The most iron content (2.790 mg/L) was observed in the extracted vermiwash by distilled water with pH 9 (**Table 8**) although there were no differences between this vermiwash and the extracted vermiwash by DTPA on iron content. When the pH of the extraction system is changed, the quantities of micronutrients extracted by chelating agents are likely to be influenced by a number of interacting factors including: (1) the amounts and forms of micronutrients initially present in vermiwash, (2) the generally decreasing availability of Zn, Cu, Fe and Mn with increasing pH due to adsorption-precipitation reactions, (3) the dispersion of organic matter and the formation of stable soluble organic matter - metal complexes as the pH reaches neutrality, and (4) the pH-dependent stability of metal chelates and secondary interactions among various metals for chelate sites (Haynes and Swift 1983).

50% of the dissolved organic matter in liquid media is made up of humic substances that influenced pH, alkalinity, chemistry and element biological availability. They can be divided in to three components according to their solubility: humic acids, fulvic acids and humin. The dissolved humic substances including humic acid and fulvic acid were only removed from water with reducing acidity to below 2 and precipitated (International Humic Substances 2007). Perhaps it can increase iron content in the extracted vermiwash by alkaline solution modified with increasing humic substances in this condition (Arancon *et al*. 2007). The results are in accordance with Rosa *et al*. (2002) study. However, Pant *et al*. (2009) reported a decrease in iron content of vermiwash with pH increase.

The extracted vermiwash by DTPA (1.934 mg/L) and 1/3 DTPA (2.328 mg/L) solutions had the highest Zinc content (**Table 8**) that was nearly the same as that found Rosa *et al*. (2002). In peat, the quantities of Zn, Cu and Mn extracted with 0.005 M DTPA or EDTA behaved irregularly as the pH was increased. The strong complexing ability of organic matter may have contributed to such trends (Haynes and Swift 1983). Also, soil pH influenced growth and reproduction of soil microorganisms (Chaoui *et al*. 2003), but the type of extractor did not significantly differ on microbial population (**Table 8**). Chaoui *et al*. (2003) reported that Microbial biomass in the earthworm cast treatments did not significantly differ from the control soil in the four final (days 43, 50, 57 and 70) sampling dates. However, soil respiration rates for the vermiwash treatments were significantly different and the most respiration activity (2.746 mg CO_2/ml.day) was found in the produced vermiwash from DTPA solution (**Table 8**). The different respiration rates in the vermiwash treatments in calong with no difference in biomass could be due to the presence of different classes of microorganisms in the casts which might have a different respiration to biomass ratio (Chaoui *et al*. 2003). The higher

Table 9 effect of substrate and extractor on coliform population (number/ml) in vermiwashes.

Substrate \ Extractor	Mature VC	Immature VC	Earthworm
Distilled water with pH=7	0	3.5×10^5	0.02×10^5
Distilled water with pH=5	0	0.045×10^5	0
Distilled water with pH=9	0	0.26×10^5	0
DTPA solution	0	12×10^6	0
1/3 DTPA solution	0	3.5×10^6	0.02×10^5

VC: vermicompost

respiration rate in the extracted vermiwash from DTPA might be due to higher nutrient content in this vermiwash. Production of CO_2 usually increases with pH when pH is less than 7 and decreases with pH at soil pH beyond 7 (Kowalenko and Ivarson 1978). Emission of CO_2 decreases by 18% at pH 8.7 and 83% at pH 10.0 as compared with pH 7.0 (Rao and Pathak 1996).

One of the main problems associated with the vermicomposting process is the presence of human pathogens, the levels of which can restrict the use of vermicompost as an organic fertilizer or its disposal by landfill. Unlike composting, vermicomposting is a mesophilic process (<35°C), so substrates do not undergo thermal stabilization that can eliminate pathogens (Aira *et al*. 2011). Nevertheless, it is known that vermicomposting may reduce the levels of different pathogens such as *Escherichia coli*, *Salmonella enteriditis*, total and faecal coliforms, helminth ova and human viruses in different types of wastes (Monroy *et al*. 2009; Edwards 2011). Parthasarathi *et al*. (2007) found that earthworms did not reduce the numbers of *Klebsiella penumoniae* and *Morganella morganii*, whereas other pathogens such as *Enterobacter aerogenes* and *Enterobacter cloacae* were completely eliminated. It has obviously been shown that vermicomposting reduced total coliform numbers (**Table 9**). Moreover, the reduction was mainly due to earthworm digestive processes, which eliminated 100% of total coliforms, probably due to a combination of its own earthworm digestive abilities. They include fine grinding of cells and several enzymes related to the degradation of bacterial cell wall (Monroy *et al*. 2008, 2009; Edwards 2011).

CONCLUSION

The type of substrate and extractor were significantly different in terms of chemical characteristic, nutrient content and respiration rate and did not influence microbial population in the final vermiwash. It is concluded that the type of substrate and the extractor influence nutrient content of the final product. So it could be used as an efficient biofertilizer in organic farming and the chemical composition of vermiwash could be obtained according to the extractor type and substrate. Since distilled water acts better than DTPA solution and almost no significant difference was found between DTPA solution and distilled water, it is suggested that application of DTPA solution for producing vermiwash is not necessary. Also, depending on the purpose of vermiwash application, distilled water could be used with acidity or alkalinity pH. The most nutrient content observed in the vermiwash produced from IV is coliform population of this substrate. Studying the effect of different kinds of vermiwash on plants growth and yield for the selection of the best is suggested.

ACKNOWLEDGEMENT

The authors thank Dr. Jaime A. Teixeira da Silva for improving the required style.

REFERENCES

Aira M, Gómez-Brandón M, González-Porto P, Domínguez J (2011) Selective reduction of the pathogenic load of cow manure in an industrial-scale continuous-feeding vermireactor. *Bioresource Technology* **102**, 9633-9637

Alef K, Nannipieri P (1995) *Methods in Applied soil Microbiology and Biochemistry*, Academic Press, London, 608 pp

American Public Health Association (APHA) (1995) 3030E nitric acid digestion. In: *Standard Methods* (20th Edn), American Public Health Association, Washington, DC

Arancon N, Edwards A, Dick R, Dick L (2007) Vermicompost tea production and plant growth impact. *Biocycle* **48**, 51

Berg B, Hannus K, Popoff T, Theander O (1982) Changes in organic-chemical components during decomposition, Long-term decomposition in a Scots pine forest I. *Canadian Journal of Botany* **60**, 1310-1319

Byzov BA, Khomyakov NV, Kharin SA, Kurakov AV (2007) Fate of soil bacteria and fungi in the gut of earthworms. *European Journal of Soil Biology* **43**, 149-156

Chaoui H, Zibilske LM, Ohno T (2003) Effect of earthworm casts and compost on soil microbial activity and plant nutrient availability. *Soil Biology and Biochemistry* **35**, 295-302

Crawford JH (1983) Composting of agricultural wastes. A review. *Process Biochemistry* **2**, 14-18

Edwards CA, Arancon NQ, Greytak S (2006) Effects of vermicompost teas on plant growth and disease. *Biocycle* **47**, 28-31

Edwards CA, Arancon NQ, Sherman R (2011) *Vermiculture Technology: Earthworms, Organic Wastes and Environmental Management*, CRC Press, Boca Raton, pp 249-261

Eisenhauer N, Straube D, Johnson EA, Parkinson D, Scheu S (2009) exotic ecosystem engineers change the emergence of plants from the seed bank of a deciduous forest. *Ecosystems* **12**, 1008-1016

Gholizadeh A (2000) Comparative of zink extractors of rice stuck on the soil of northern Iran, and recommend appropriate extractor for soil. MSc thesis, University of Tehran, 121 pp (in Persian)

Grobe K (2003) California landscape contractor calls it compost tea time. *BioCycle* **44**, 26-27

Gutierrez-Miceli FA, García-Romero RC, Rincón-Rosales R, Abud-Archila M, Oliva-Llaven MA, Guillen-Cruz MJ, Dendooven L (2008) Formulation of a liquid fertilizer for sorghum (*Sorghum bicolor* (L.) Moench) using vermicompost leachate. *Bioresource Technology* **99**, 6174-6180

Haynes RJ, Swift RS (1983) An evaluation of the use of DTPA and EDTA as extractants for micronutrients in moderately acid soils. *Plant and Soil* **74**, 111-122

Hosomi M, Sudo R (1986) Simultaneous determination of total phosphorus in fresh water samples using persulfate digestion. *International Journal of Environmental Studies* **27**, 267-275

Ingham E (2005) *The Compost Tea Brewing Manual* (5th Edn), Latest Methods and Research Soil Food Web Inc., Corvallis, OR, 79 pp

International Humic Substances Society (2007) Available online: www.humicsubstances.org

Kaviraj, Sharma S (2003) Municipal solid wastes management through vermicomposting employing exotic and local species of earthworm. *Bioresource Technology* **90**, 169-173

Keeling AA, McCallum KR, Beckwith CP (2003) Mature green waste compost enhances growth and nitrogen uptake in wheat (*Triticum aestivum* L.) and oilseed rape (*Brassica napus* L.) through the action of water-extractable factors. *Bioresource Technology* **90**, 127-132

Kowalenko CG, Ivarson KC, Cameron DR (1978) Effect of moisture content, temperature and nitrogen fertilization on carbon dioxide evolution from field soils. *Soil Biology and Biochemistry Journal* **10**, 417-423

Lindsy WL, Norvell WA (1978) Development of a DTPA soil test for zinc, iron, manganese, and copper. *Soil Science Society of America Journal* **42**, 421-428

Moez Ardalan M, Svaqby Gh R (2001) *Soil Fertility Management for Sustainable Agriculture*, Tehran University, 387 pp (in Persian)

Monroy F, Aira M, Domínguez J (2008) Changes in density of nematodes, protozoa and total coliforms after transit through the gut of four epigeic earthworms (Oligochaeta). *Applied Soil Ecology* **39**, 127-132

Monroy F, Aira M, Domínguez J (2009) Reduction of total coliform numbers during vermicomposting is caused by short-term direct effects of earthworms on microorganisms and depends on the dose of application of pig slurry. *Science of the Total Environment* **407**, 5411-5416

Nath G, Singh K, Singh DK (2009) Chemical analysis of vermicomposts / vermiwash of different combinations of animal, agro and kitchen wastes. *Australian Journal of Basic and Applied Sciences* **3**, 3671-3676

Page AL, Miller RH, Keney DR (1982) *Methods of Soil Analysis Part 2 (Chemical and Micribiological Properties)* (2nd Ed, Vol 9), Agronomic Monographs, A.S.A. and SSSA Madison, Wisconsin, 1692 pp

Pant A, Radovich TJK, Hue NV, Talcotte ST, Krenek K (2009) Vermicompost extracts influence growth, total carotenoids, phenolics and antioxidant activity in pak choi (*Brassica rap* acv. Bonsai, Chinensis group) grown under two fertilizer regimes. *Journal Science Food and Agriculture* **89**, 2383-2392

Parthasarathi K, Ranganathan LS, Anandi V, Zeyer J (2007) Diversity of microflora in the gut and casts of tropical composting earthworms reared on different substrates. *Journal of Environmental Biology* 2**8**, 87-97

Parthasarathi K, Balamurugan M, Ranganathan LS (2008) Influence of vermicompost on the phisico-chemical and biological properties in different types of soil along with yield and quality of the pulse crop-blackgram. *Iranian Journal Environmental Health Science Engineering* **5**, 51-58

Valembois P, Roch P, Lassègues M, Cassand P (1982) Antibacterial activity of the haemolytic system from the earthworms *Eisenia foetida andrei*. *Journal of Invertebrate Pathology* **40**, 21-27

Rao DLN, Pathak H (1996) Ameliorative influence of organic matter on biological activity of salt affected soils. *Arid Soil Research and Rehabilitation* **10**, 311-319

Recycled Organics Unite (2003) *Buvers's Guide for Recycled Organics Products*. Information sheet 6-5. Recycled organics product categories and standards. Recycled Organic Unite, The University of New South Wales Sydney Australia 1466 internet publication: www.recycledorganics.com/

Rosa AH, Rocha JC, Burba P (2002) Extraction and exchange behavior of metal species in therapeutically applied peat. *Talanta* **58**, 969-978

Ryan J, Estefan G, Rashid A (2001) *Soil and Plant Analysis Laboratory Manual*, ICARDA, Aleppo, Syria, 172 pp

Salardini AA (2002) *Soil Fertility* (1st Edn, Vols I-VI), University of Tehran Press (in Persian), 428 pp

Sharma SD, Devi M, Kumar P, Bhardwaj SK, Raj H (2011) Potential use of bioorganic nutrient source dynamics on cropping behavior, soil properties, and quality attributes of apricot. *Communications in Soil Science and Plant Analysis* **42**, 1656-1674

Sparks DL (1996) *Analysis Chemical Methods Part 3*, American Society of Agronomy-Soil Science Society of America, SSSA, Madison, WI, 1264 pp

Suthar S (2010) Evidence of plant hormone like substances in vermiwash: An ecologically safe option of synthetic chemicals for sustainable farming. *Ecological Engineering* **36**, 1086-1092

Tripathi G, Bhardwaj P (2004) Decomposition of kitchen waste amended with cow manure using epigeic species (*Eisenia fetida*) and an anecic species (*Lampito mauritii*). *Bioresource Technology* **92**, 215-218

Vasanthi K, Chairman K, Savarimuthu Michael J, Kalirajan A, Ranjit Singh AJA (2011) Enhancing bioconversion efficiency of the earthworm *Eudrilus Eugeniae* (Kingberg) by fortifying the filter mud vermibed using an organic nutrient. *Online Journal of Biological Sciences* **11**, 18-22

Venkatesh RM, Eevera T (2008) Mass reduction and recovery of nutrients through vermicomposting of fly ash. *Applied Ecology and Environmental Research* 6, 77-84

Welke SE (2005) The effect of compost extract on the yield of strawberries and the severity of *Botrytis cinerea. Journal of Sustainable Agriculture* **25**, 57-68