STUDY OF SOME BIOLOGICAL PARAMETERS OF THE RED CALIFORNIAN EARTHWORM *EISENIA FOETIDA* (SAVIGNY, 1826) IN VERMICOMPOST FOLLOWING THE APPLICATION OF WOOD VINEGAR

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Abstract. This study was carried out with the purpose to assess some biological parameters of the red Californian worm (*Eisenia foetida*) in vermicompost, to which two types of wood vinegar, manufactured from poultry manure (A) and nutshell (B), have been applied. The study was implemented under laboratory conditions with Coincidence Parcels Trial Layout and 5 repetitions in 2018. Profile Analysis Technique was used in the in the investigation of wood vinegar types and the effect of various doses on the weight gain and number of worms on a weekly basis, while Factorial ANOVA was used in the investigation of their effects on cocoon numbers. The Profile Analysis proved that the interaction between Week × Dose × Vinegar type was important in regards to both the weight and number of worms (P = 0.00). The Factorial ANOVA carried out to investigate the effects of the vinegar type and doses on the cocoon number showed that the Dose × Vinegar type interaction was important (P = 0.00). Therefore, the effects of the vinegar type on the number and weight of worms differed depending on the dosage. In general, it was noted that 3%- and 5% dosages of vinegars had positive effects on *Eisenia foetida*, while the dosage of 100% had toxic effects. Based on the findings, it is possible that wood vinegars have a potential use with various purposes including bio-pesticides or worm manure.

Keywords: agricultural, bio-economy, bio-pesticides, cocoon, pyrolysis liquids, worm

Introduction

Although earthworms are not abundant in number, they are probably significant factors for sustaining soil productivity in various ways (Edwards and Bohlen, 1992). These living beings are accepted as important indicators of chemical toxicity in the soil ecosystem (Bustos-Obregón and Goicochea, 2002; Yasmin and D'Souza, 2010). Wood vinegar (WV) is a liquid produced through carbonization (pyrolysis liquids) that has been found to had been produced as early as the time of the Neanderthals (Tiilikkala et al., 2010; Cai et al., 2012). Kim et al. (2008) stated with reference to Jang that WV is a clear liquid containing 80-90% water with the rest being water-soluble organic compounds exceeding 200 in number. Jothityangkoon et al. (2008) stated that the main components of WV were acetic acid paired with organic acids and phenolic compounds, alkanes, alcohols and esters. This product is used as manure, insect repellant or organic fungicide in agriculture. Furthermore, there are studies showing that WV has effects that improve the soil and promote plant growth. As examples of such studies, Jothityangkoon et al. (2008), Yin (2008), Baimark and Niamsa (2009), Velmurugan et al. (2009), Chalermsan and Peerapan (2009), Oramahi and Yoshimura (2013), Hagner (2013), Namlı et al. (2014), Koc et al. (2017), De Souza Araújo et al. (2018) and Oramahi et al. (2018) have performed studies concerning the antifungal activities of WV. Studies of Yatagai et al. (2002), Oramahi and Yoshimura (2013) are notable on the issue of anti-termite effects of WV. It was stated by the studies of Shi (2003),

Jothityangkoon et al. (2008), Yin (2008), Rakmai (2009), Cai et al. (2012) that WV affects plant growth. Regarding the antibacterial effectiveness of WV, positive remarks of Chalermsan and Peerapan (2009), Eric et al. (2012), Duan et al. (2016), Yang et al. (2016), Chukeatirote et al. (2018) and De Souza Araújo et al. (2018) can be noted. Koç et al. (2018) have found significant relations between treatments with WV and nematodes in the soil. Shi (2003) and Rakmai (2009) have obtained findings indicating the activities of WV that increase the edaphon and the number of bacteria in the root area of plants. There are also studies by Inoue et al. (2000), Yatagai et al. (2002), Jothityangkoon et al. (2008), Diba et al. (2009), Kiarie-Makara et al. (2010), Pangnakorn et al. (2011), Wititsiri (2011), Hagner (2013), Oramahi and Yoshimura (2013) explaining the insecticide or insect-repellant effects of WV. Cai et al. (2012) have reported that WV promotes the development of reactive oxidative species (ROS)sensitive mutant worms, the lifetime of worms is prolonged and their body size increased. Mu et al. (2003) have stated that WV is a good resource for organic agriculture and is widely used in Japan, both in agriculture and daily life. Kim et al. (2008) have stated that the annual production of WV is around 14.000 tons. Hagner (2013) has stated in his study that there is little information on the toxic effects of WV, while Rakmai (2009) stated referring to Ikeshima that this substance has no toxic effects on humans or animals. Tiilikkala et al. (2010) have reported that pyrolysis liquids have the potential of replacing pesticides and synthetic chemicals in biocide form in the future, and this is based on bio-economic knowledge and depends on its development. As seen in the studies cited above, is it clear that WV has multiple effects.

This study has been carried with the purpose of determining some biological parameters of the red Californian earthworm (*Eisenia foetida*) in vermicompost after application of two types of wood vinegar obtained from poultry manure (A) and nutshell (B).

Materials and methods

This study was carried out under laboratory conditions in 2018. The vermicompost required for the experiments was obtained from 100% cow manure, and cocoons were obtained through reproduction from stock cultures of Eisenia foetida individuals. Wood vinegar (WV), was obtained from a firm developing WV products from poultry manure (A) and nutshell (B) using a gasification machine and bio-coal (Naml1 et al., 2014). Vinegar obtain from nutshell (B) is light brown in color, and is characterized by a burning smell. Vinegar obtained from poultry manure (A) however, is darker in color as compared to vinegar B, is more consistent and has a stronger odor. The study was implemented using the Coincidence Parcels Trial Layout with 5 repetitions (Fig. 1). After placing 100 g vermicompost in 300 cm³ plastic containers, the solution was treated with WV using a 10-mL injector. One percent, 3%, 5%, 10%, 25% and 100% WV doses were used in the treatments, and only tap water was injected into the control group. WV treatment was performed only once. Following these procedures, 10 cocoons were placed in each container. The ambient temperature of the study was kept between 20 and 29 °C and the humidity was kept between 70 and 90% (Gunadi et al., 2002). With the purpose of maintaining the ambient humidity, 10 mL tap water was administered to the samples periodically with 2 day intervals. Live weight, number of cocoons laid, and the number of young individuals coming out of cocoons were determined weekly until the young individuals laid cocoons themselves. Profile

Analysis was used during the investigation of the effects of different doses and vinegar types on weekly body weight gain and number of worms (Mendeş et al., 2007). Factorial ANOVA was used to investigate the effects of the factors mentioned above on the number of cocoons, and Tukey's Multiple Comparison Test was used in determining the different groups (Winer et al., 1991; Mendeş, 2012). The abovementioned analyses were carried out using the SPSS (Ver. 21.0) statistical package programs.



Figure 1. Photo of the experimental culture

Results

Findings related to the weights of worms

Profile Analysis results for WV(A) and WV(B) are presented in *Figures 2* and *3*, respectively. Results of Profile Analysis indicated that the Week × Dose × Vinegar type interaction is significant (Wilks' Lambda = 0.253; F = 3.61; P = 0.00). Meaning that the effect of vinegar type on weekly body weight gain of worms varied based on the doses (*Figs. 2* and *3*; *Table 1*). When only week 5 is taken into consideration in comparison with the controls, treatments with WV (B) have not resulted in much difference between the body weights of worms (*Fig. 3; Table 1*). However, treatments with WV (A) 1%, 25% and 100% had negative effects while treatments with 3% and 5% had positive effects (*Fig. 2; Table 1*).

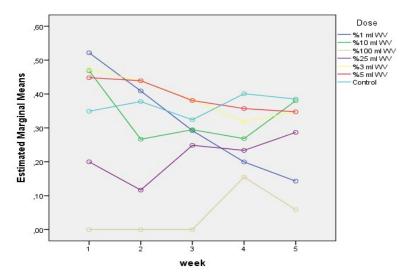


Figure 2. Profile analysis for investigating the effect of WV(A) on weekly body weight gain of worms based on doses

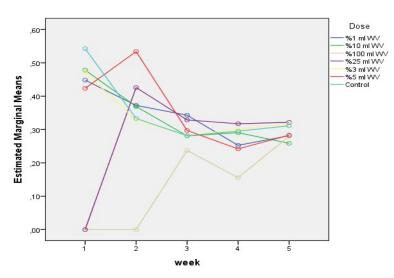


Figure 3. Profile analysis for investigating the effect of WV (B) on weekly body weight gain of worms based on doses

Week	WV dose	Vinegar type	Mean	Std. deviation	N	Week	WV Dose	Vinegar type	Mean	Std. deviation	N
		А	0.522	0.131	5			А	0.409	0.076	5
	1%	В	0.448	0.134	5		1%	В	0.372	0.114	5
		Total	0.485	0.131	10			Total	0.390	0.093	10
		А	0.473	0.043	5			А	0.438	0.145	5
	3%	В	0.471	0.112	5		3%	В	0.329	0.120	5
		Total	0.472	0.080	10			Total	0.384	0.138	10
		А	0.448	0.024	5			А	0.439	0.055	5
	5%	В	0.424	0.050	5		5%	В	0.534	0.072	5
		Total	0.436	0.039	10			Total	0.487	0.078	10
	10%	А	0.469	0.625	4		10% 25% 100%	А	0.267	0.309	4
		В	0.479	0.099	5	- W4		В	0.369	0.036	5
w3		Total	0.474	0.080	9			Total	0.324	0.198	9
M	25%	А	0.200	0.187	5			А	0.117	0.162	5
		В	0.000	0.000	5			В	0.426	0.042	5
		Total	0.100	0.163	10			Total	0.271	0.198	10
		А	0.000	0.000	4			А	0.000	0.000	4
	100%	В	0.000	0.000	5			В	0.000	0.000	5
		Total	0.000	0.000	9			Total	0.000	0.000	9
		А	0.349	0.058	5			А	0.378	0.128	5
	Control	В	0.543	0.081	5		Control	В	0.333	0.059	5
		Total	0.446	0.122	10			Total	0.356	0.097	10
		А	0.359	0.191	33			А	0.302	0.209	33
	Total	В	0.338	0.232	35		Total	В	0.338	0.169	35
		Total	0.348	0.212	68			Total	0.320	0.189	68
		А	0.292	0.175	5			А	0.200	0.274	5
w5	1%	В	0.343	0.049	5	9m	1%	В	0.253	0.025	5
		Total	0.318	0.124	10			Total	0.226	0.185	10

Table 1. Descriptive statistics for the body weight of worms

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		Α	0.383	0.063	5			Α	0.318	0.035	5
	3%	В	0.282	0.050	5		3%	В	0.301	0.043	5
		Total	0.333	0.075	10			Total	0.309	0.038	10
		А	0.381	0.080	5			А	0.357	0.024	5
	5%	В	0.297	0.019	5		5%	В	0.242	0.027	5
		Total	0.339	0.070	10			Total	0.300	0.065	10
		А	0.295	0.057	4			Α	0.268	0.179	4
	10%	В	0.281	0.023	5		10%	В	0.291	0.034	5
		Total	0.287	0.039	9			Total	0.281	0.113	9
		Α	0.249	0.172	5			Α	0.234	0.136	5
	25%	В	0.329	0.077	5		25%	В	0.317	0.059	5
		Total	0.289	0.133	10			Total	0.276	0.108	10
		Α	0.000	0.000	4		100%	А	0.155	0.232	4
	100%	В	0.237	0.154	5			В	0.156	0.213	5
		Total	0.132	0.166	9			Total	0.155	0.207	9
		Α	0.324	0.075	5			А	0.401	0.034	5
	Control	В	0.281	0.049	5		Control	В	0.295	0.021	5
		Total	0.303	0.064	10			Total	0.348	0.062	10
		Α	0.282	0.153	33			А	0.280	0.164	33
	Total	В	0.293	0.074	35		Total	В	0.265	0.095	35
		Total	0.288	0.118	68			Total	0.272	0.132	68
		А	0.143	0.198	5			Α	0.287	0.055	5
	1%	В	0.282	0.022	5		25%	В	0.322	0.059	5
		Total	0.212	0.152	10			Total	0.304	0.057	10
		Α	0.347	0.066	5			Α	0.059	0.117	4
	3%	В	0.317	0.016	5		100%	В	0.280	0.256	5
Γw		Total	0.332	0.048	10	Lw7		Total	0.182	0.227	9
3		Α	0.347	0.021	5	≥		А	0.385	0.016	5
	5%	В	0.283	0.018	5		Control	В	0.311	0.034	5
		Total	0.315	0.039	10			Total	0.348	0.046	10
		Α	0.380	0.023	4]		Α	0.282	0.144	33
	10%	В	0.259	0.033	5		Total	В	0.293	0.095	35
		Total	0.313	0.070	9			Total	0.288	0.120	68

Findings on the number of worms

Profile Analysis results for WV(A) and WV(B) are presented in *Figures 4* and *5*, respectively. Results of Profile Analysis indicated that the Week × Dose × Vinegar type interaction is significant (Wilks' Lambda = 0.05; F = 7.83; P = 0.00). Meaning that the effect of vinegar type on weekly number of worms varied based on the doses (*Figs. 4* and *5*, *Table 2*).

It can be noted that treatments with 1%, 25% and 100% WV (A) have negative effects on the number of worms in comparison with controls, while 3% and 5% doses have positive effects (*Fig. 4; Table 2*). It was also seen that the 100% dose of WV (B) had negative effects in comparison with the controls, while treatments with 3%, 5% and 10% WV had positive effects (*Fig. 5; Table 2*).

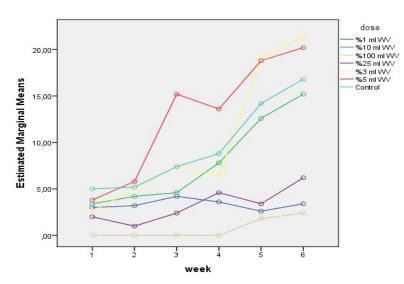


Figure 4. Profile analysis for investigating the effect of WV (A) on weekly number of worms based on doses

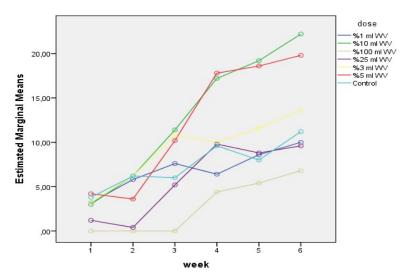


Figure 5. Profile analysis for investigating the effect of WV (B) on weekly number of worms based on doses

Week	WV Dose	Vinegar type	Mean	Std. deviation	N	Week	WV Dose	Vinegar type	Mean	Std. deviation	Ν
		А	3.000	1.581	5			А	3.200	1.304	5
	1%	В	3.200	1.304	5		1% 3% 5%	В	5.800	0.836	5
		Total	3.100	1.370	10			Total	4.500	1.716	10
		А	2.600	0.894	5	w3		А	5.000	1.000	5
w2	3%	В	3.200	0.447	5			В	6.200	0.837	5
		Total	2.900	0.738	10			Total	5.600	1.075	10
	5%	А	3.800	1.304	5			А	5.800	0.837	5
		В	4.200	0.447	5			В	3.600	1.140	5
		Total	4.000	0.943	10			Total	4.700	1.494	10

Table 2. Descriptive statistics for number of worms

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	application of wood vinegar
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	1		2 400	1 (70	-	1	1		1 4 2 0 0 1	0.447	1-
	100/	A	3.400	1.673	5	-	100/	A	4.200	0.447	5
	10%	B	3.000	1.414	5		10%	В	6.200	1.095	5
		Total	3.200	1.476	10			Total	5.200	1.316	10
		A	2.000	1.414	5			A	1.000	1.000	5
	25%	B	1.200	0.447	5		25%	В	0.400	0.548	5
		Total	1.600	1.075	10			Total	0.700	0.823	10
		A	0.000	0.000	5			A	0.000	0.000	5
	100%	В	0.000	0.000	5		100%	В	0.000	0.000	5
		Total	0.000	0.000	10			Total	0.000	0.000	10
		A	5.000	1.000	5			A	5.200	0.447	5
	Control	В	3.800	0.447	5		Control	В	6.200	1.304	5
		Total	4.400	0.966	10			Total	5.700	1.059	10
		A	2.829	1.855	35			A	3.486	2.215	35
	Total	В	2.657	1.589	35		Total	В	4.057	2.754	35
		Total	2.743	1.717	70			Total	3.771	2.497	70
		А	4.200	1.924	5			A	3.600	2.191	5
	1%	В	7.600	0.894	5		3%	В	6.400	1.342	5
		Total	5.900	2.283	10			Total	5.000	2.261	10
		А	7.400	0.548	5			A	6.400	0.894	5
	3%	В	10.800	1.304	5			В	10.000	1.225	5
		Total	9.100	2.025	10			Total	8.200	2.150	10
		А	15.200	1.789	5			A	13.600	3.050	5
	5%	В	10.200	2.049	5		5%	В	17.800	1.924	5
		Total	12.700	3.199	10			Total	15.700	3.268	10
	10%	А	4.600	1.517	5	w5	10%	Α	7.800	3.493	5
		В	11.400	1.342	5			В	17.200	1.304	5
w4		Total	8.000	3.829	10			Total	12.500	5.543	10
~		А	2.400	0.548	5		25%	Α	4.600	2.881	5
	25%	В	5.200	0.447	5			В	9.800	3.962	5
		Total	3.800	1.549	10			Total	7.200	4.264	10
		А	0.000	0.000	5			Α	0.000	0.000	5
	100%	В	0.000	0.000	5		100%	В	4.400	2.608	5
		Total	0.000	0.000	10			Total	2.200	2.898	10
		А	7.400	0.548	5			А	8.800	1.304	5
	Control	В	6.000	0.707	5		Control	В	9.600	3.647	5
		Total	6.700	0.949	10			Total	9.200	2.616	10
		А	5.886	4.708	35			Α	6.400	4.558	35
	Total	В	7.314	3.917	35		Total	В	10.742	5.271	35
		Total	6.600	4.359	70			Total	8.571	5.358	70
		А	2.600	3.578	5			Α	3.400	3.507	5
	1%	В	8.600	1.817	5		1%	В	10.000	1.414	5
		Total	5.600	4.142	10			Total	6.700	4.296	10
		А	19.000	1.871	5			Α	21.400	2.074	5
9m	3%	В	11.600	1.517	5	w7	3%	В	13.600	2.191	5
ы		Total	15.300	4.218	10			Total	17.500	4.577	10
		А	18.800	2.588	5			Α	20.200	1.924	5
	5%	В	18.600	2.608	5		5%	В	19.800	3.033	5
		Total	18.700	2.452	10			Total	20.000	2.404	10
	10%	А	12.600	4.278	5		10%	Α	15.200	2.280	5

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		В	19.200	1.643	5		В	22.200	2.168	5
		Total	15.900	4.630	10		Total	18.700	4.244	10
		А	3.400	2.408	5		А	6.200	1.095	5
	25%	В	8.800	2.168	5	25%	В	9.600	2.191	5
		Total	6.100	3.573	10		Total	7.900	2.424	10
		А	1.800	1.483	5		А	2.400	2.074	5
	100%	В	5.400	3.050	5	100%	В	6.800	3.899	5
		Total	3.600	2.951	10		Total	4.600	3.748	10
		А	14.200	0.836	5		А	16.800	1.304	5
	Control	В	8.000	2.121	5	Control	В	11.200	3.633	5
		Total	11.100	3.604	10		Total	14.000	3.916	10
		А	10.343	7.550	35		А	12.229	7.807	35
	Total	В	11.457	5.447	35	Total	В	13.314	5.890	35
		Total	10.900	6.559	70		Total	12.771	6.887	70

Findings related to cocoon numbers

Factorial ANOVA was used to analyze the data set. Results of ANOVA showed that the Dose × Vinegar type interaction is significant (P = 0.00). Therefore, the effect of vinegar type on number of cocoons varied based on the doses (*Table 3; Fig. 6*). Upon comparison of WV (A) treatments with the controls, doses of 3% and 5% exerted positive effects on the number of cocoons, while treatments of 1%, 25% and 100% doses had negative effects. A smaller numbers of cocoons was produced under treatments with WV (B) compared to the controls (*Table 3*).

WV Dose	Vinegar type	Mean	Grouping	Std. error	Ν
	А	0.000	b	0.000	5
1%	В	1.600	a	1.342	5
	Total	0.800		1.229	10
	А	4.000	а	0.707	5
3%	В	0.400	b	0.548	5
	Total	2.200		1.989	10
	А	4.000	a	0.707	5
5%	В	0.000	b	0.000	5
	Total	2.000		2.160	10
	А	3.200	a	1.304	5
10%	В	1.600	a	0.548	5
	Total	2.400		1.265	10
	А	1.800	a	0.447	5
25%	В	0.800	b	0.837	5
	Total	1.300		0.823	10
	А	0.600	a	0.894	5
100%	В	0.000	a	0.000	5
	Total	0.300		0.675	10

Table 3. Descriptive statistics and Tukey multiple comparison test for the number cocoons

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Control	А	3.200	а	0.836	5
	В	2.600	b	0.548	5
	Total	2.900		0.738	10
	А	2.400	а	1.684	35
Total	В	1.000	b	1.111	35
	Total	1.700		1.582	70

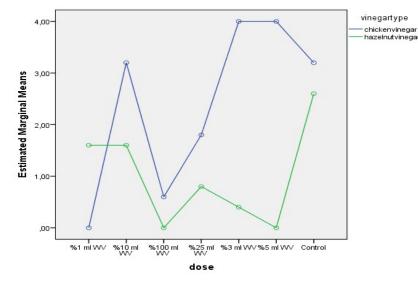


Figure 6. Changes in the number of cocoons based on dose and vinegar type

Discussion

Chemical pesticides and fertilizers used in agricultural activities pollute the environment, damage biodiversity and exert negative effects on human health. This situation can be eliminated through the use of alternative methods such as organic pesticides and manures. Wood vinegar (WV) is an organic product that can be degraded in a short time produced from plant and animal waste. Treatments with WV (A, B) have been found to have effects on the body weight, number of worms and number of cocoons depending on the type of vinegar, dose and the week of measurements. Of these vinegar types, WV (A) is thought to have exerted positive effects on the numbers of worms and cocoons particularly in doses of 3% and 5% (Table 3), while WV (B) has positive effects particularly on the number of worms in 3%, 5% and 10% doses (Table 2). These results are consistent with the findings of Cai et al. (2012) indicating that WV exerts positive effects on the development, lifetime and body size of worms. Furthermore, the fact that WV has positive effects on plants and other living beings in the soil is supported by the findings of Shi (2003), Jothityangkoon et al. (2008), Yin (2008), Rakmai (2009) and Cai et al. (2012). However, studies indicating the negative effects of a 1% dose on worms has been attributed to either an adverse factor in the test apparatus or to some toxic effect. Generally, the 100% dose and partially 25% dose of WV have been determined to exert negative effects on the biological parameters of worms. These results are supported by the findings of Inoue et al. (2000), Yatagai et al. (2002), Jothityangkoon et al. (2008), Yin (2008), Baimark and Niamsa (2009),

Velmurugan et al. (2009), Chalermsan and Peerapan (2009), Diba et al. (2009), Kiarie-Makara et al. (2010), Pangnakorn et al. (2011), Wititsiri (2011), Eric et al. (2012), Oramahi and Yoshimura (2013), Hagner (2013), Namlı et al. (2014), Duan et al. (2016), Yang et al. (2016), Koc et al. (2017), De Souza Araújo et al. (2018), Oramahi et al. (2018) and Chukeatirote et al. (2018), agreeing that WV can exert bactericide, fungicide, anti-termite and insecticide effects. However, it can easily be seen from the examination of figures and tables of these studies that the effect of types and doses of WV differ weekly. It is estimated that this can be attributed to the statements of Kim et al. (2008) and Jothityangkoon et al. (2008) in their respective studies, stating that vinegar consists of water-soluble substances exceeding 200 in number and the main components are acetic acid paired with organic acids, phenolic acids, alkanes, alcohol and esters.

Conclusion

As a result of this study we can conclude that types and doses of wood vinegar can have positive effects on certain parameters of *Eisenia foetida*. It is thought that the 3% and 5% doses in particular exert positive effects on worms, while the dose of 100% has toxic effects. Vinegar types with all their characteristics may have a potential use in agriculture, or more precisely in organic agriculture and worm manure production.

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