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SHREDDED PAPER AS FOOD FOR EISENIA FETIDA: CHANGES IN SOIL AND IN SEVERAL BIOLOGICAL PARAMETERS

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ABSTRACT

Earthworms of species *Eisenia fetida* (Savigny 1826) (red wiggler worm), are known as eaters of a large range of organic matter, being considered from this point of view real solvers of several issues regarding the wastes management. This study intended to show that paper wastes (copy paper) can be eaten by earthworms *Eisenia fetida* and thus this type of waste can be managed by introducing it into the diet of this earthworm species. The objectives of the study were two: 1) to determine the relationship existing between this food type and several biological parameters of this earthworm species (survival, weight, and prolificacy); 2) to establish the relation existing between this earthworm's mortality, weight and prolificacy and several soil properties (the concentration of the total nitrogen, available potassium, and available phosphorus). The experiment lasted three months and has been carried out as a laboratory (microcosmos) experiment. The results showed that copy paper represents an adequate food for earthworms *Eisenia fetida* (no mortality was recorded in the experimental pots as compared to controls, and the earthworm's prolificacy increased, on average with 65.16% versus control ($p < 0.05$). Feeding the earthworms *Eisenia fetida* with shredded paper is appropriate to increase the prolificacy of this species, parameter which positively is correlated with the values of pH, mobile phosphorus and mobile potassium of the soil, and to increase the concentrations of phosphorus and potassium in the soil, but this last aspect requires further investigations, because probably this is an effect on short time. The content of mobile phosphorus of soil increased with 98.56% in the soil containing earthworms fed with shredded paper versus control (soil without earthworms) ($p < 0.01$, $p < 0.05$). The parameter mobile potassium of the soil increased with 15.57% as compared with the soil with unfed earthworms ($p < 0.05$). In this study, feeding earthworms *Eisenia fetida* with shredded paper was not a way to obtain significant increases of the value of total nitrogen in soil. The body weight of the earthworms fed with shredded paper was found to be positively correlated to the content of total nitrogen of the soil, and negatively correlated to the parameters pH, mobile phosphorus, and mobile potassium. The characteristic prolificacy was found to be positively correlated to the parameters pH, mobile phosphorus, mobile potassium, and no significant negative correlations were found for this biological parameter and the soil factors. There was performed statistical

processing of data using Spearman and Kendall Correlations, One Way ANOVA, and Pearson Correlation ($p<0.01$, $p<0.05$).

Keywords: mortality, prolificacy, *Eisenia fetida*, shredded paper, soil

INTRODUCTION

The wastes of paper represent an ecological and economical issue of nowadays as other organic wastes produced by the human society. Solving this issue using biotechnologies became a priority in the environmental concerns of the present [1-10; 12].

The potential of living organisms to neutralize organic wastes is often exploited for several reasons, especially because represents a cheap and rapid solution, environmental friendly [3-10]. Many studies investigated different species of earthworm in vermicomposting different types of organic wastes [1-10]. Genus *Eisenia* has been investigated in composting of paper waste too. In order to determine the growth and reproduction rates of earthworms *Eisenia andrei* fed with paper sludge, Elvira et al. (1998) found that the number of earthworms increased between 22- and 36-fold and total biomass increased between 2.2- and 3.9-fold, concluding that these sludge could reduce the costs related with the exclusive use of different types of farm wastes as feed for earthworms [3]. In order to determine the optimum C/N ratio for converting waste paper to nutrient-rich manure with minimum toxicity using earthworms *Eisenia fetida*, Ravindran and Mnkeni (2016) revealed that this species considerably degraded the organic waste and significantly improved the bioconversion and nutrient value of all treatments, depending on time during the vermicomposting process [4]. Generally, a good strategy for the management of paper waste using earthworms *Eisenia fetida* is to combine the paper wastes with chicken manure [4] or cattle dung [5]. There was found that earthworm *Eisenia fetida* accelerates the rate of mineralization and converts the paper wastes (from paper cups) into compost with elements able to support the growth of crop plants [6-8]. Moreover, this species can remove heavy metals from paper mill wastewater sludge [9] and helps in fast conversion of toxic paper mill sludge into a soil conditioner in 100 days [5].

Other studies noted the performance of other earthworm species (*Eudrilus eugeniae, Drawida willsi, Lampito mauritii, Perionyx excavatus*) in terms of vermicast output per unit feed, production of offspring, and increase in worm biomass in compostation of paper waste [10].

The objectives of this study were two: 1) to determine the relationship existing between this food type and several biological parameters of this earthworm species (survival, weight, and prolificacy); 2) to establish the relation existing between this earthworm's mortality, weight and prolificacy and several soil properties (the concentration of the total nitrogen, available potassium, and available phosphorus).

MATERIALS AND METHODS

The researches were carried out as a chamber experiment (microcosmos) organized at the University of Agricultural Sciences and Veterinary Medicine of Banat „King Michael the Ist of Romania" from Timişoara, Timiş County, Romania. The experiment lasted three months. The microclimate conditions were: 20°C ± 2°C environmental

temperature and the natural photoperiod characteristic to the region (45°45'N21°14'E) for the research period (November – January). The microcosmos experiment has been carried out in control boxes (*Eisenia fetida* not fed with shredded paper) and in test boxes (*Eisenia fetida* fed with shredded copy paper) and was established in three replicates. The soil used within experiment was a cambic chernozem according to FAO System (2.97% content of total organic carbon) and it was collected from the first 30 cm of topsoil (cleaned off by plants and other vegetal rests and without earthworm cocoons). In each box there were introduced 1660 g of soil and ten earthworms *Eisenia fetida* (adults, with their body weight ranging between 4.49 – 5.33 g, without significant differences between weight values). In each test box was added nutritive substrate as following: 3 g of shredded copy paper (80 $g \cdot m^{-2}$) moisturized with 20 ml water for 1 hour, then mixed with 40 g of experimental soil. The nutritive substrate has been added in the test boxes at six days from the moment when earthworms have been introduced in boxes (from the start of the experiment) and after 45 days from the start of the experiment. The moisture content was controlled at a value between 70 and 80%. The moisture content has been established by drying a substrate sample at 105°C for 48 h. The following parameters were monitored in the control boxes and in the test boxes: body weight of earthworms at the start and at the end of the experiment, respectively; earthworm survival (as earthworms found alive) at the start and at the end of the experiment; earthworm prolificacy (hatched larvae) at the end of the experiment; the variation of values for the following parameters of soil: pH, total nitrogen, available potassium, and available phosphorous. The number of earthworms and hatchlings were counted by hand sorting. The parameters of soil (pH, total nitrogen, available potassium, and available phosphorous) were established using the following methodology: the pH values have been established by potentiometric method in aqueous suspension (pH_{H2O}), ratio soil:solution was 1:2.5; the content in total nitrogen of soil – by the Kjeldahl method; the mobile (plant available) phosphorus and potassium of soil have been determined by spectrophotometry and flame spectrometry methods, respectively, by the Egner-Riehm-Domingo method [11]. The statistical processing was realized using the software SPSS (Statistical Package for the Social Sciences).

RESULTS AND DISCUSSION

In the Table 1 is listed data about several biological parameters of the species *Eisenia fetida* investigated within experiment. There can be observed no mortality at the end of the experiment of earthworms fed with shredded paper versus 16.17% mortality in control, where the earthworms received no food. The biomass of earthworms decreased within experiment as a result of earthworm translocation into the experimental soil which had less humidity as compared to the initial environment. Other researchers revealed increases of the biomass of earthworm species *Eisenia fetida* during the vermicompostation of paper wastes. Ravindran and Mnkeni 2016 showed that this increase depends on time factor, possibly due to a greater reduction of toxic substances in these wastes [4]. The prolificacy of earthworm fed with shredded paper increased with 65.16% as against control after three months of experiments (paired sample T-test, $p = 0.023$).

Table 1. Mortality, body weight, and prolificacy of the species *Eisenia fetida* within experiment

Experimental variant	Mortality		Body weight		Prolificacy
	Earthworm number at the start of experiment	Earthworm number at the end of experiment	Earthworm weight at the start of experiment (g)	Earthworm weight at the end of experiment (g)	Number of hatched larvae after 3 months of experiment
Control	10	8.33 ± 2.08	5.07 ± 0.51	2.09 ± 0.63	6.66 ± 4.04*
Standard Error	0	1.47	0.36	0.44	2.33
Shredded paper	10	10	5.21 ± 0.18	2.92 ± 0.26	11.00 ± 3.00*
Standard Error	0	0	0.10	0.15	1.73

*Paired Sample T-test ($p < 0.05$): $t(2) = -6.500$, $p = 0.023$.

Among the analysed parameters of the soil, the value representing the content of the mobile phosphorus of the soil significantly increased with 98.56% in the soil containing earthworms fed with shredded paper as compared with control (soil without earthworms) (One-Way ANOVA, $p < 0.01$; Paired Sample T-test, $p < 0.05$). A significant increase was also found for the parameter mobile potassium of the soil, respectively with 15.57% as compared with the soil with unfed earthworms (Paired Sample T-test, $p < 0.05$) (Table 2). Because the experiment lasted only three months, it is necessary to survey these findings in time. Ravindran and Mnkeni 2016 found that, during the vermicompostation of paper wastes using the species *Eisenia fetida,* the total N, total P, and total K concentrations increased with time while total carbon, C/N ratio, electrical conductivity, and heavy metal content gradually decreased with time [4].

Table 2. Values for several parameters of the experimental soil

Experimental variant	Indicator	Values
Soil without *Eisenia fetida*	pH (pH units)	7.89 ± 6.08^{4}
	Nt (%)	0.15 ± 2.51^{1}
	P ($mg \cdot kg^{-1}$)	18.14 ± 0.13^{2}
	K ($mg \cdot kg^{-1}$)	220.66 ± 6.11
Soil with *Eisenia fetida*	pH (pH units)	$7.79 \pm 6.02^{3,4}$
	Nt (%)	0.21 ± 1.52^{1}
	P ($mg \cdot kg^{-1}$)	$36.02 \pm 5.07^{2,5}$
	K ($mg \cdot kg^{-1}$)	188.33 ± 20.00^{6}
Soil with *Eisenia fetida* fed with shredded paper	pH (pH units)	8.06 ± 0.03^{3}
	Nt (%)	0.18 ± 0.02
	P ($mg \cdot kg^{-1}$)	28.41 ± 2.15^{5}
	K ($mg \cdot kg^{-1}$)	217.66 ± 27.09^{6}

[1]One-Way ANOVA ($p < 0.05$): $F(1.4) = 12.462$, $p = 0.024$; Paired Sample T-test ($p < 0.05$): $t(2) = -6.000$, $p = 0.027$;

[2]One-Way ANOVA ($p < 0.01$): $F(1.4) = 37.376$, $p = 0.004$; Paired Sample T-test ($p < 0.05$): $t(2) = -6.211$, $p = 0.025$;

[3]One-Way ANOVA ($p < 0.01$): $F(1.4) = 47.891$, $p = 0.002$; Paired Sample T-test ($p < 0.05$): $t(2) = -7.688$, $p = 0.017$;

Observations: Because for the following correlations the *p* value is slightly over 0.05, these could be considered in the interpretation of the achieved results:

[4] Paired Sample T-test ($p < 0.05$): $t(2) = 3.883$, $p = 0.060$;

[5]One-Way ANOVA ($p < 0.05$): $F(1.4) = 5.715$, $p = 0.075$; Paired Sample T-test ($p < 0.05$): $t(2) = 3.504$, $p = 0.073$;

[6]Paired Sample T-test ($p < 0.05$): $t(2) = -3.617$, $p = 0.069$.

The study showed several statistically significant correlations between the studied factors which are listed in the Table 3. Generally, the body weight of the earthworms fed with shredded paper is positively correlated to the content of total nitrogen of the soil, and negatively correlated to the parameters pH, mobile phosphorus, mobile potassium). The characteristic prolificacy is positively correlated to the parameters pH, mobile phosphorus, mobile potassium, and no significant negative correlations were found for this parameter and the soil factors.

Table 3. Correlations between body weight and prolificacy of earthworms *Eisenia fetida* fed with shredded paper and the concentrations of mobile phosphorous and potassium of the adjacent soil (Spearman and Kendall Correlations: $p < 0.01$, Pearson Correlation: $p < 0.05$)

Correlations between body weight of the earthworms fed with shredded paper and the pH of soil	Pearson's Correlation Coefficient	–1.000**
	Sig. (2-tailed)	0.05
	Spearman's Correlation Coefficient	–1.000**
	Sig. (2-tailed)	0.000
Correlations between body weight of the earthworms fed with shredded paper and the concentration of the total nitrogen of soil	Pearson's Correlation Coefficient	0.997*
	Sig. (2-tailed)	0.05
	Kendall's Correlation Coefficient	1.000**
	Sig. (2-tailed)	0.000
	Spearman's Correlation Coefficient	1.000**
	Sig. (2-tailed)	0.000
Correlations between body weight of the earthworms fed with shredded paper and the concentration of the mobile phosphorus of soil	Spearman's Correlation Coefficient	–1.000**
	Sig. (2-tailed)	0.000
Correlations between body weight of the	Pearson's Correlation Coefficient	–0.994

earthworms fed with shredded paper and the concentration of the mobile potassium of soil	Sig. (2-tailed)	0.07
	Spearman's Correlation Coefficient	–1.000**
	Sig. (2-tailed)	0.000
Correlations between the prolificacy of the earthworms fed with shredded paper and the pH of soil	Kendall's Correlation Coefficient	1.000**
	Sig. (2-tailed)	0.000
	Spearman's Correlation Coefficient	1.000**
	Sig. (2-tailed)	0.000
Correlations between the prolificacy of the earthworms fed with shredded paper and the concentration of the total nitrogen of soil	Pearson's Correlation Coefficient	0.993
	Sig. (2-tailed)	0.07
	Kendall's Correlation Coefficient	1.000**
	Sig. (2-tailed)	0.000
	Spearman's Correlation Coefficient	1.000**
	Sig. (2-tailed)	0.000
Correlations between the prolificacy of the earthworms fed with shredded paper and the concentration of the mobile phosphorus of soil	Kendall's Correlation Coefficient	1.000**
	Sig. (2-tailed)	0.000
	Spearman's Correlation Coefficient	1.000**
	Sig. (2-tailed)	0.000
Correlations between the prolificacy of the earthworms fed with shredded paper and the concentration of the mobile potassium of soil	Pearson's Correlation Coefficient	0.996*
	Sig. (2-tailed)	0.05
	Kendall's Correlation Coefficient	1.000**
	Sig. (2-tailed)	0.000
	Spearman's Correlation Coefficient	1.000**
	Sig. (2-tailed)	0.000
Correlations between body weight of the earthworms not fed with shredded paper and the pH of soil	Kendall's Correlation Coefficient	1.000**
	Sig. (2-tailed)	0.000
	Spearman's Correlation Coefficient	1.000**
	Sig. (2-tailed)	0.000
Correlations between body weight of the earthworms not fed with shredded paper and the concentration of the total nitrogen of soil	Kendall's Correlation Coefficient	1.000**
	Sig. (2-tailed)	0.000
	Spearman's Correlation Coefficient	1.000**
	Sig. (2-tailed)	0.000
Correlations between body weight of the earthworms not fed with shredded paper and the concentration of the mobile potassium of soil	Spearman's Correlation Coefficient	–1.000**
	Sig. (2-tailed)	0.000
Correlations between the prolificacy of the earthworms not fed with shredded paper and the pH of soil	Pearson's Correlation Coefficient	0.999*
	Sig. (2-tailed)	0.030
	Kendall's Correlation Coefficient	1.000**

	Sig. (2-tailed)	0.000
	Spearman's Correlation Coefficient	1.000**
	Sig. (2-tailed)	0.000
Correlations between the prolificacy of the earthworms not fed with shredded paper and the concentration of the total nitrogen of soil	Pearson's Correlation Coefficient	0.999*
	Sig. (2-tailed)	0.030
	Kendall's Correlation Coefficient	1.000**
	Sig. (2-tailed)	0.000
	Spearman's Correlation Coefficient	1.000**
	Sig. (2-tailed)	0.000
Correlations between the prolificacy of the earthworms not fed with shredded paper and the concentration of the mobile phosphorus of soil	Pearson's Correlation Coefficient	–0.999*
	Sig. (2-tailed)	0.030
	Spearman's Correlation Coefficient	–1.000**
	Sig. (2-tailed)	0.000

CONCLUSIONS

The results of this study showed that copy paper represents an adequate food for earthworms *Eisenia fetida* (no mortality was recorded in the experimental pots as compared to controls, and the earthworm's prolificacy increased, on average with 65.16% versus control (paired sample T-test, $p = 0.023$). Feeding the earthworms *Eisenia fetida* with shredded paper is appropriate to increase the prolificacy of this species, parameter which positively is correlated with the values of pH, mobile phosphorus and mobile potassium of the soil, and to increase the concentrations of phosphorus and potassium in the soil, but this last aspect requires further investigations, because probably this is an effect on short time because, although these parameters increased in the soil containing earthworms *Eisenia fetida* fed with shredded paper, their body mass decreased, which led to the conclusion that it is necessary to investigate on long time what happens with earthworm weight related to the chemical parameters of soils and also with their prolificacy. Although there was recorded a decrease of the body weight during this experiment, this was attributed to the earthworms translocation at the start of experiment into a less moisturized environment. The content of mobile phosphorus of soil increased with 98.56% in the soil containing earthworms fed with shredded paper versus control (soil without earthworms) (One-Way ANOVA, $p < 0.01$; Paired Sample T-test, $p < 0.05$). The parameter mobile potassium of the soil increased with 15.57% as compared with the soil with unfed earthworms (Paired Sample T-test, $p < 0.05$). In this study, feeding earthworms *Eisenia fetida* with shredded paper was not a way to obtain significant increases of the value of total nitrogen in soil. The body weight of the earthworms fed with shredded paper was found to be positively correlated to the content of total nitrogen of the soil, and negatively correlated to the parameters pH, mobile phosphorus, and mobile potassium. The characteristic prolificacy was found to be positively correlated to the parameters pH, mobile phosphorus, mobile potassium, and no significant negative correlations were found for this biological parameter and the soil factors.

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