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Dynamic of Organic Matter and Available Nutrients in Heavy Metal Contaminated Soil Under the Effect of Eisenia Andrei Earthworms

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Abstract

Heavy metal contamination is an important environmental problem which couldaffect the durability and fertility of soils. For the enhancement of soil properties in mine soils, many technologies can be used. In this study, we have assessed the efficacity of vermeremdiation (The use of earthworms for remediation). In this context, this study investigate the effect of earthworms Eisneia andrei on physicochmical properties of soils. For this purpose, six polymetllaic contaminated soils from Jebel Ressass mine (ranged from the most contamianted one A to control one F) were incubated during 14, 28 & 60 days in presence and absence of earthworms Eisenia Andrei. Organic matter, available phosphorous, potassium and magnesium and active limestone were assessed in each incubation point. Our results schowed that earthworms increased organic matter content especially for soil A. Also, a rise was observed in the mineral content and active limestone within 14 days of addition. This cork showed the efficiency of earthworms on the amelioration of chemical properties of soils from mine site

Keywords: Heavy metal; Mine soil; Earthworms; Chemical properties; Soil

Introduction

Heavy metals are considered as a natural constituent of the earth crust. Their sources in the environment can originate from natural and anthropogenic activities. Anthropogenic activities such as mining, smelting, industrial, and agricultural use increase the abundance of heavy metals in the environment [1-4]. They can be toxic to living organisms at very low levels of exposure. They did not only lead to soil contamination, but also affect food production, quality and safety [5-6].

Indeed, soil pollution by persistent metals concerns large areas at the global scale, particularly in industrial and mining environments [7]. Indeed, numerous industrial or mining sites, often abandoned, are now in urban areas and are therefore likely to cause environmental and health risks to surrounding populations [8-10]. These sites are sources of fine particles enriched with pollutants leading to contamination of soils and plants [11-13].

In Tunisia, heavy metal pollution presents a critical problem affecting the ecosystem functioning and soil fertility. Jebel Ressas mining activities started in 1892 and was used for the extraction of lead and zinc. The activity was intense for over forty years and was interrupted in 1959. Over decades, mining activities have accumulated large wastes which covered vast hectares of the agricultural farmlands and had deteriorated the quality and fertility of soils [14-15]. Up to now, few studies have been conducted on this site in order to rehabilitate its biological activity [16-17].

In this context and for the remediation of heavy metal soils, many strategies of decontamination were proposed. However, the biological methods play a crucial role in thoroughly cleaning up the contaminants in soils [18]. For that, we use bioremediation for the stabilization/mineralization of heavy metals to reduce their bioavailability. The biological agents can be simple organisms (bacteria, plants, etc.). Compared to physicochemical methods conventionally used to decontaminate soils but which lead to a sharp decline in fertility and productivity, bioremediation is considered as a friendly environmental technology.

Besides, earthworms are ecosystem engineers, driving soil structure and nutrient dynamics and their importance in soil ecosystems has long been recognized. By feeding on litter and soil, burrowing and releasing casts, earthworms change soil porosity, bulk density, water infiltration, nutrient mineralization, gas emissions, organic carbon stabilization and plant productivity [19-22]. Moreover, in many studies, earthworms had been proposed as actors for soil bioremediation, denominated Verme remediation [23-24]. Their mechanisms for soil detoxification are multiples. Earthworms could influence metal bioavailability in soils through the mixing and comminution of soil particles and by the humic materials and detritus contained in the earthworm gut [25-26]. Moreover, earthworms could have direct effect on heavy metals via uptaking it into their tissue, which was proved by several studies [27-29] The other mechanism was the process of mineralization and humification under the interaction between earthworms and microorganisms, which could also affect heavy metal transformation in soils [30-32].

For this purpose, we aimed in this present work to assess if earthworms can change physicochemical properties of soil originated from mine site. The final objective is to ameliorate soil fertility in heavy metal contaminated soils.

Materiel and methods

Sites and samples collection

The present work was conducted on Jebel Ressass mining. Soils chosen in this work are characterized by a gradient of heavy metal concentration [Table 1]. They ranged from the most contaminated one (A) to the less contaminated one (F) constating the site control. Soils from each site were sampled from a depth of 30cm. Then, they were homogenized and transferred in the laboratory. Soils were air-dried and sieved through a 2mm screen and they were conserved until use.

Table 1: Characteristics of soils samples collected from Jebel Ressas sites.

Parameters	Site A	Site B	Site C	Site D	Site E	Site F
Land use	Mine Spoils	Olive Tree	Olive Tree	Olive Tree	Olive Tree	Olive Tree
Cu (ppm)^b	14.25±2.18	9.81±0.78	4.58±0.27	1.27±0.11	1.78±0.16	1.98±0.14
Fe (ppm)^b	1.28±0.44	1.75±0.05	1.31±0.11	1.25±0.24	1.53±0.11	2.6±0.11
Mn (ppm)^b	306±21.40	285±15.12	186±12.35	217±17.54	244±31.21	413±31.25
Zn (ppm)^b	42400±245	6690±452	2550±254	753± 54	323±21	174±12
Pb (ppm)^b	14500±278	2490±321	985±87	374± 44	173±12	116±15
Cd (ppm)^b	184.00±10.25	27.10±2.31	13.20±1.45	1.59±0.24	1.54±0.09	0.67±0.01

Animals used in the experiment

Mature earthworms (with clitellum two months adult) of the species E. Andrei were obtained from a local synchronized culture in our laboratory and weighed individually (average earthworm weight 500mg).

Experimental procedure

The experimental design was conducted as a completely randomized factorial design with three factors. The first factor is the level of contamination constituting by the six soils, the second factor is the period of incubation: 14, 28&60 days and the third factor had two levels (introduction or not of E. Andrei). Three replicates per treatment were carried out, making a total of 108pots. The experience was undertaken under controlled conditions. One kilogram of dried and sieved (<2mm) soil was placed in polyethylene pots. Twenty mature earthworms were randomly placed in each pot. The half of pots being without earthworms. Soil moisture was maintained at 60% of water holding capacity using distilled water and temperature at 25°C. This was maintained through the experience. Each pot was covered with fine nylon to prevent soil loss and stop earthworm escape. At the end of the exposure period, soils (three replicates per treatment) were conserved at ambient temperature until their analyses.

Analytical procedures

The same set of pulverized soil samples were analyzed using an automated elemental analyzer to determine the total C content. As the soil contains no inorganic carbon, the total carbon estimated is OC itself. The soil samples were weighed and encapsulated in tin foils and were introduced to the furnace at 95 OC of the elemental analyser (Leco Corporation, USA, Truspec CN) and flushed with oxygen for complete combustion. The carbon containing compounds were oxidized to CO2 and separated from all other oxides and lead to the infra-red gas analyser for estimation. The instrument system was ABRAHAM 49 calibrated with soil standards supplied by Leco Corporation, USA each time the estimations was carried out.

Plant available phosphorous (P) and potassium (K) were determined by calciumacetate-lactate (CAL) extraction (ÖNORM L1087).

Statistical results

The R software was used for all the statistical analysis in this paper. The normality of the distribution was carried out using the Shapiro-Wilk test. For multiple comparisons, a parametric one-way analysis of variance (ANOVA) was performed with Tukey's test. The student test was used to compare the soil

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enzymatic activities in the presence or absence of earthworms. To compare the evolution of physicochemical properties of soils, principal component analyses (PCAs) were performed using the R software and the package ADE4TkGUI.

Results

Effect of earthworms Eisenia Andrei on organic matter of Jebel Ressass soils

The organic matter content in Jebel Ressass soils has changed significantly following the incorporation of Eisenia andrei earthworms. Indeed, it increased only in the case of soil A where means raised from 1.7% ±0.14 initially to 2.06% ±0.05 and 1.936% ±0.09, respectively after 28 & 60 days of incubation. In the other soils, despite the increase noted after 14 days for soils B and D and after 28 days for soils C and E, the content of organic matter decreased after 60 days of incubation. In soil F, SOM decreased by a range of 40%, which represents the largest reduction comparing to all soils. Moreover, in comparison to soils incubated without earthworms, the main difference was observed in soils A and E after 30 days [Figure 1].

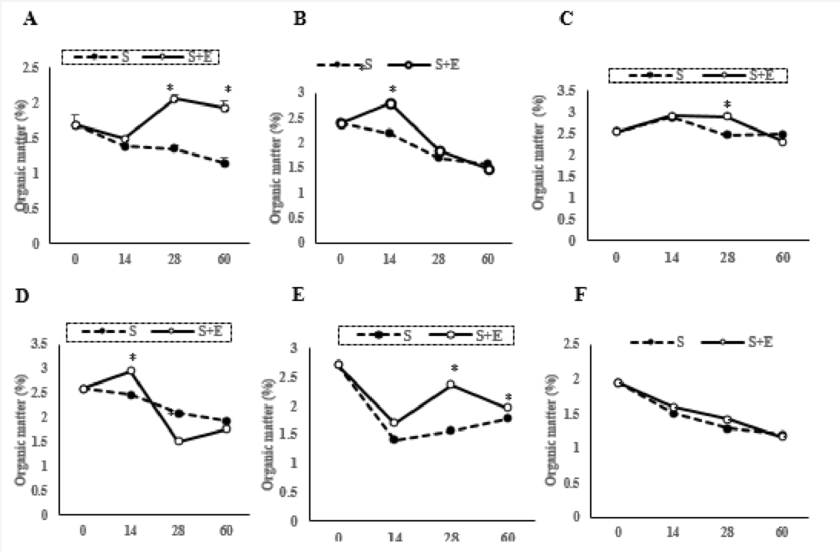

Figure 1: Change on organic matter content (%) in six polymetallic contaminated soils from Jebel Ressas sites during 14, 28 and 60 days and in presence (S+E) and absence (S) of earthworms Eisenia andrei. Significative difference between soil in presence and absence of earthworms days and in presence (S+E) and absence (S) of earthworms Eisenia andrei. Significative difference between soil in presence and absence of earthworms.

Effect of earthworms on phosphorus content of Jebel Ressass soils

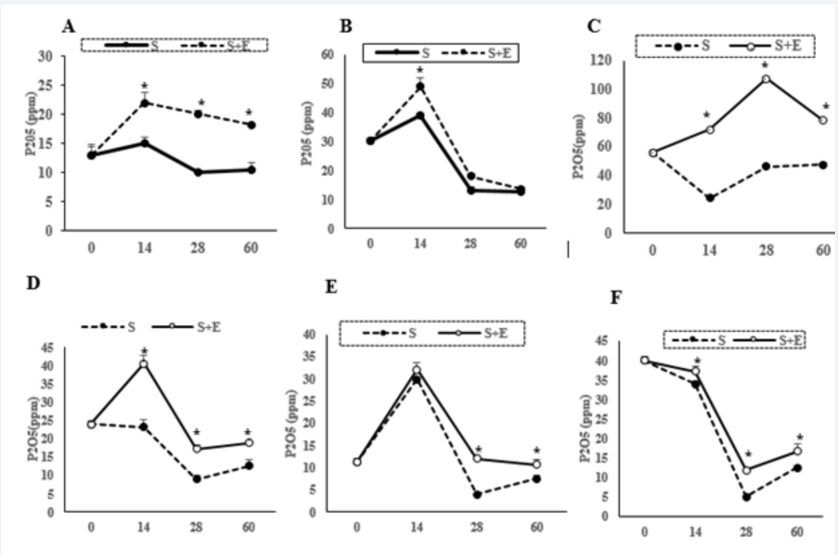

Figure 2: Change on phosphorous content (ppm) in six polymetallic contaminated soils from Jebel Ressas sites during 14, 28 and 60, in presence (S+E) and absence (S) of earthworms Eisenia andrei. Significative difference between soil in presence and absence of earthworms.

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The incorporation of Eisenia Andrei worms into the six soils of Jebel Ressass had provoqued changes in available phosphorus content [Figure 2]. These changes had the same trend in all soils, except for the control soil F. The results showed an increase in the first two points of the kinetics (14 days for soils A, B, D and E and 28days for soil C) followed by a crucial decrease after 60 days of incubation. In contrast, the available phosphorus content in the control soil (F) decreased at the different points of the kinetics. In addition, the effect of earthworms was mainly observed in soils A, D and C where the P2O5 content was twice more important in soils with earthworms compared to control soils, successively after 14 days. incubation for the first two soils and 28 days for the third soil.

Effect of earthworms on potassium content of Jebel Ressass soils

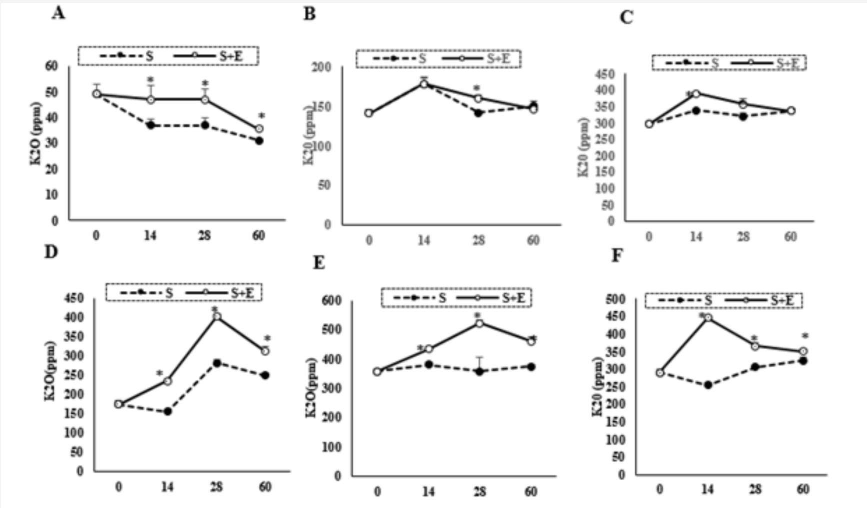

Figure 3: Change on phosphorous content (ppm) in six polymetallic contaminated soils from Jebel Ressas sites during 14, 28 and 60, in presence (S+E) and absence (S) of earthworms Eisenia andrei. Significative difference between soil in presence and absence of earthworms.

The assimilable potassium content K2O changed in Jebel Ressass soils [Figure 3] under the effect of earthworms Eisenia Andrei. Indeed, the trend observed in soils B, C, D, E & F consists of an increase firstly followed by a decrease after 60 days of incubation. The largest increases were observed in soils D and F with values of 402.04 ±7.54&446 ±4 ppm, respectively after 28 and 14 days for both soils. Soil A is the only one where the K2O content didn't increase after animals' incorporation. In addition, the earthworm effect was most observed in soils A, D, E and F where the K2O content was significantly higher compared to control soils (without earthworms). On the other hand, soil E had the highest levels of K2O in all kinetic points.

Effect of earthworms on magnesium content of Jebel Ressass soils

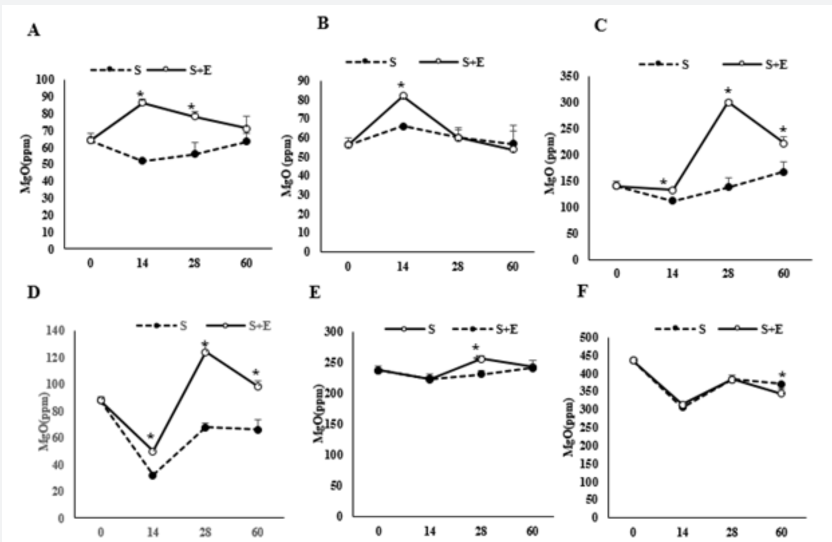

Figure 4: Change on magnesium content (%) in six polymetallic contaminated soils from Jebel Ressas sites during 14, 28 and 60, in presence (S+E) and absence (S) of earthworms Eisenia andrei. Significative difference between soil in presence and absence of earthworms.

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Incorporation of E. Andrei earthworms into Jebel Ressass soils affected available magnesium levels [Figure 4]. Results showed that soils A and B had the same pattern of response which is demonstrated by an increase after 14 days of incubation followed by a decrease which leads to a lower content than the initial one (0 day) for soil B. Magnesium content in soils C, D, E had changed throughout the kinetics in a similar way. Indeed, a decrease was noted after 14 days of incubation followed by an increase reaching the maximum level observed for these soils after 30 days. However, after 60 days of incubation, a decrease was observed for the magnesium content of these soils. On the other hand, in the control soil, a decrease in the Mg2+ content was observed throughout the kinetics points. Despite this decrease, the content remains higher than that of the others. The most important change, observed under the effect of the earthworms Eisenia Andrei, was noted in the soil D after 28 days of incubation where the Mg2+ content was twice as great in the soils containing earthworms by compared to controlled soils (without earthworms).

Effect of earthworms Eisenia andrei on active limestone

The active limestone content has changed following the incorporation of earthworms into Jebel Ressass soils [Figure 5]. These changes were mainly observed in soils B, C, D and E where the content is 1.5 times higher in soils with earthworms compared to control soils (without earthworms). On the other hand, in soils A, B, C and D an increase in the concentration of active limestone was observed and the maximum peak was noted for soils A and C after 14 day of animal incorporation and in soils B and D after 28 days. However, in these soils the active limestone content decreased after 60 days in except of soil D. In the least contaminated soils E and F, a decrease according to the different incubation times was observed.

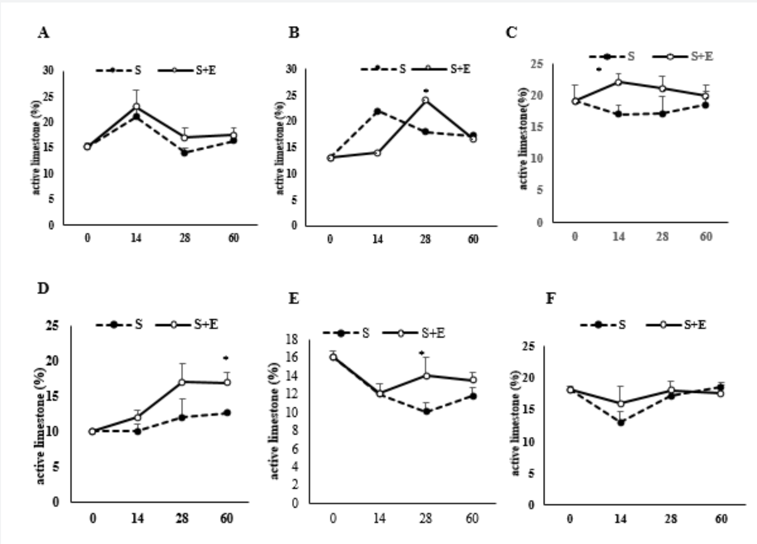

Figure 5: Change on active limestone (%) in six polymetallic contaminated soils from Jebel Ressas sites during 14, 28 and 60, in presence (S+E) and absence (S) of earthworms Eisenia andrei. Significative difference between soil in presence and absence of earthworms.

Principal analysis component of the effect of earthworms on soil physoco-chemical properties

The evolution of physico-chemical properties of soil after 14, 28 and 60 days of Eisenia andrei addition is represented in Figure 6. PCA represented in Figure 6(a) illustrates change in physico-chemical parameters after 14 days of incubation. Axis 1 which represent 37,02% of variability sepparate soil A and B from E and F one. Axis 2 presenting 25,19 % of variability differenciates soil A from C. Results schowed a change adter erathworms addition, however, this effect was espceillay observed in soils A, B, C and D.

After 28 days of incubation, the axes 1 and 2 of PCA [Figure 6(b)] present respectively 24.50 and 36, 30% of the total variance. The first axis differentiates soils A and B from E and F one. The second axis separates C from soils D, E and F. The PCA showed a change in physico-chemical parameters under the effect of earthworms in all soils, except in the case of soil D. However, the most significant variation was noted in the most polluted soils A, B and C.

After 60 days of incubation (figure 6(c)), the axes 1 & 2 of the PCA represent respectively 26,18 and 34% of the total variance. The first axis differentiates soils A and B from soils E and F. The second axis separates soil E from C one. The PCA demonstrates a change in physicochemical parameters, in the presence of earthworms in all soils. However, the most important changes was observed in soils A, B, C and D.

Discussion

Beneficial roles of earthworms on soil fertility, nutrient cycling and plant growth have been commonly observed. However, few studies have focused on this effect in heavy metal polluted soils. In this study, soils originated form mine soils, Jebel Ressass, were incubated with earthworms Eisenia

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Andrei in view of assessing its effect on soil chemical fertility. Our results demonstrated firstly an important increase on organic matter in soil A which is the most contaminated one, especially after 60 days of incubation. We can then hypothesize then that earthworms in mine soils require an acclimation period before starting to produce effects. Moreover, such results create interesting perspectives on the use of earthworms in the rehabilitation of mining cuttings. However, after 60 days of incubation, organic matter decreased in the other soils under the effect of earthworms. This result is consistent with the work of which demonstrated the important role of the digestive tract of earthworms in the decomposition of organic matter. Indeed, earthworms feed on organic matter, break it down and release the minerals to be available to plants. Consequently, organic matter content decrease. However, this result is contradictory with the work of which observed an increase in organic matter content under the effect of earthworms in a Pb artificially contaminated soil.

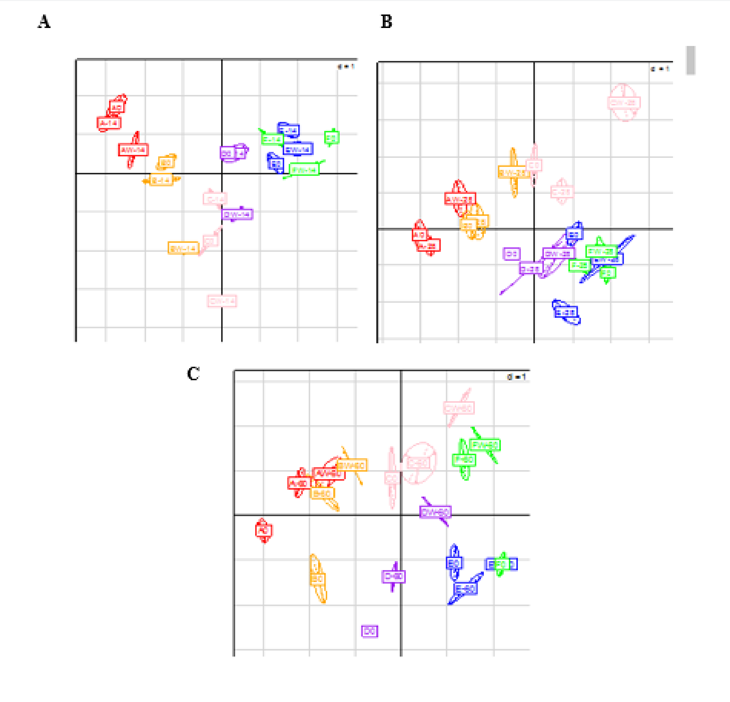

Figure 6: PCA analysis of chemical properties dynamics of six polymetallic contaminated soils from Djebel Ressas site after 14 (A), 28 (B) and 60 (C) days of incubation with and without Eisenia Andrei introduction. Sample name: First letter represents the soil, W means with worms, and the number is the incubation time. Soil A: red ellipses, soil B: orange ellipses, soil C: purple ellipses, sol D: cyan ellipses, sol E: blue ellipses, and soil F: green ellipses.

About the availability of nutrients P205, K20 & Mg0, our results demonstrated that earthworms burrowing earthworms increased the availability of nutrients in the first point of incubation and secondary decreased it after 60 days of incubation. Here, two assumptions can explain this result. First, when worms are introduced, these latter degrade organic matter and release nutrients, leading to an increase on their bioavailability. After 60 days of incubation, the organic matter content decreased causing the reduction of the bioavailability of nutrients. Second, the intensity of organic matter degradation and the release of nutrients are modified by change on the structure of soil bacterial communities after earthworm's addition. This was demonstrated in a previous work. Therefore, a change in the structure of soil bacterial communities will induce variations in the mode of digestion of organic matter.

On the other hand, the positive effect of worms on soil chemical fertility, specifically the increase in bioavailability of nutrients has been widely demonstrated. However, very few studies have investigated the effect of earthworms on nutrient availability for soils contaminated with ETMs. In this context showed, in the case of soils contaminated with Pb, that Pontoscolex corethrurus increased the bioavailability of the three major elements N, P and K. Earthworms can ameliorate chemical fertility for soils by burrowing and casting, with the formation of aggregates allowing easy penetration of water and air. Also, earthworm activity modifies soil pH, a key chemical factor affecting bioavailability of nutrient elements and heavy metals in soils. Another explication for variation on nutrients availability can be the change observed on soil enzymes activities under the effect of earthworms. Indeed, soil enzymes constitute the principal key in C, N and P cycling. In previous study, Eisenia Andrei had increase enzymes activities in soils. These results can generate an important change on chemical properties of soils.

Conclusion

Lessons learnt from this study implicate that earthworms may be applied for soil management. Further, earthworms can

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be used in bioremediation of soils contaminated by various pollutant such as heavy metals.

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