Original Research Article

THE IMPACT OF AN EARTHWORM DIGESTION ON THE CHEMICAL COMPOSITIONS OF VARIOUS CLAY MINERALS FROM SOILS

ABSTRACT

Aims: The impact of living organisms activities in the soil may generate several chemical and mineralogical changes. The objectif of this study is to estimate the impact of earthworms on the chemistry of soil minerals as the clay minerals.
Study design: An experiment was carried integrating earthworms and clay minerals. The modifications due to this interaction between minerals and organic matter are discussed.

Place and Duration of Study: Institut des Sciences de la Terre et de l'Environnement (UdS/CNRS), Université de Strasbourg, Strasbourg, France. 8 weeks of experiment.
Methodology: *Lumbricus terrestris* earthworm species was used for digestion of illite and various smectite-type minerals, with or without peat moss, during a laboratory experiment at a constant temperature of 20°C during 8 weeks and under artificial 12 hour-light and 12 hour-dark cycles, for each digestion cycle.

Results: The chemical elements extracted from smectites by the earthworm digestion appear to be smectite-type dependent. The behavior of K and radiogenic ^{40}Ar is more dictated by the type of digested smectite than by the digestion process itself.

The ^{87}Sr/^{86}Sr ratio of the acid leachates of the nontronite and peat mixture, before and after digestion, suggests that: half of the digested matter corresponds to digestive action of the earthworms, with the second half from food. Digested clay material represents up to 30% and the peat moss the remainder of the mixtures.

Conclusion: This study highlighted the role of organic compounds which it is generated by earthworms activities in transfer of elements to surface waters and soil fluids.

Keywords: ***Ar and Rb-Sr tracing clay minerals, Earthworm digestion, Elemental variations, Impact on illite and various smectites; K-peat moss***

1. INTRODUCTION

Earthworms have an important activity in soil ecosystems; they especially help improve the soil structure, as well as its chemical and biological properties. They are especially important in no-till hosting environments by stimulating the air and water movements. Their activity mixes the organic and inorganic fractions of the nearby environment along their digestive tract by decomposing these two major components of their guts along interactions with micro-organisms including bacteria, algae, protozoa, fungi and others [1]. Therefore, earthworms are major decomposers of dead organic matter by a fragmentation action. They make a major contribution to recycling the nutrients of soils by generating tons of casts per acre each year.

The time taken for food to be digested by earthworms varies from 3 to 4 hours in species such as for *Eisenia fetida*, to about 12 to 20 hours in others such as *Lumbricus terrestris* [2]. The individual volumetric impact of earthworm rejections is minimal, but some claimed that earthworm casts could amount to layers of 0.25 mm to 2 mm thick per year in some areas, providing reasonable annual turnovers of soil by earthworm activity [3, 4, 5]. Earthworms have also feeding preferences: the vertically burrowing species *Lumbricus terrestris* feeds large amounts of plant remains, while *Apporectodea Caliginosa*, a horizontally burrowing species, searches also for mineral components.

In fact, as mixtures of soil minerals and organics digested by earthworms are changing, the chemical and mineralogical modifications of minerals digested by the earthworms is of interest for the evaluation of overall chemical budgets of soils. Based on such behaviors and budgets, one may not only understand processes by which various major minerals of soils are modified, but also get a broader insight into the organic activities that influence the chemistry of the surface water that might be consumed by humans. In this context, the present study is an attempt based on an experimental approach towards a more decrypted interaction between living organisms and their surrounding mineral and organic compounds. More specifically, it evaluates the impact of earthworms digestion on various clay minerals mixed or not with an organic component, as clays represent a major active constituent of soils. Within this objective is also a better identification of the process by which fragile soil minerals are modified, for instance by earthworm activities.

Most studies on earthworm activities in soils being essentially applied to bulk topsoils to delineate chemical changes from an experimental approach [6, 7, 8], they do not allow identification of a detailed action on individual mineral components. Their scopes range from soil stability and porosity to availability of nutrients such as N, P, C, K and Ca to a lesser degree, to a potential contribution of various micro-organisms and enzymes along the decomposition of the guts, to the absorption of metals in the body tissues as a determination of the level of metal contamination of soils, and to the production of nitrous oxide by microbial activities in the guts [9]. In fact, studies on earthworms represent a way to gain a more detailed insight into the global weathering processes just below the soil surface, as well as some understanding of the influence of living organisms on the chemistry of soil minerals and therefore on that of the associated fluids by quantifying their impact on major components of bulk soils such as the clay minerals, which is the frame of the present attempt.

2. MATERIALS AND METHODS

Selected reference clay minerals were grind and reduced to an average size of <65 μm and were leached with de-ionized water, 8 grams of the minerals being used for each experiment. Also thoroughly leached by de-ionized water, peat moss was the plant material added to the minerals for an examination of the organic/inorganic combination. Also grind to <65 μm, 2 grams of this peat were used for the experiments. Four earthworm individuals of *Lumbricus terrestris* were added to each mineral-organic mixture for the successive experiments completed in the laboratory in a hood with filtered air, at a constant temperature of 20°C during 8 weeks and under artificial 12 hour-light and 12 hour-dark cycles, for each digestion cycle. The various components were systematically mixed in 15 cm high by 12 cm diameter plastic containers. The recovered guts were leached after the experiments with 100 ml of de-ionized water and the leachates were recovered after each experiment for an evaluation of the interaction. The analytical techniques included X-ray diffraction for the visualization of the changes of the mineral structures, as well as inductively coupled plasma-atomic emission spectrometry (ICP-AES) and inductively coupled plasma-mass spectrometer (ICP-MS) for the elemental contents of the minerals, their residues after digestion, and of the leached guts. A K-Ar isotopic tracing was also applied to the different minerals before and after the earthworm digestion, which is somehow new in such experimental conditions. The reason for its use here was because it allows a precise evaluation of the K and radiogenic ^{40}Ar contents and variations and, therefore, of any discrete change in the mineral structure resulting from events like a worm digestion. Potassium is bound in the interlayer positions of the clay minerals examined here, while the radiogenic ^{40}Ar is known to be only squeezed in them, not tied by an identified connexion [9, 10]. ^{87}Sr/^{86}Sr tracing was also compiled to evaluate a further tool for numerating the changes that could have occurred in the mineral and organic solids digested by the earthworms.
The K contents were measured by flame spectrometry with a basic precision better than 1.5wt% for K amounts higher than 0.5wt%. This routine analytical accuracy was improved here by an initial

screening of the aliquots that yield K contents below this 0.5% limit in framing each result between two internal standards set below and above the screened contents, the final contents being determined by interpolating the three values at a precision estimated at 0.5wt%. The basic analytical technique follows the procedure of [11]. The use of the K-Ar isotopic dating and ^{87}Sr/^{86}Sr tracing methods were used to help controlling the behavior of radiogenic ^{40}Ar that is not keyed in K-rich mineral structures like those studied here, but only squeezed, and tracking the origins of Sr by analyzing the digested solids and their leachable components. These behaviors were thought to provide further *Lumbricus terrestris*, information about the impact of the earthworm digestion on the minerals and the organics. The methods were applied, in turn, to quantify the removable elements of the clay minerals before and after earthworm digestion.

The Ar extractions were completed in a glass line, following the method of [12] with an overnight preheating of the samples at 80°C under vacuum to remove as much as possible the atmospheric Ar adsorbed on the particles during preparation and handling. The accuracy of the mass spectrometer was checked weekly by measuring the international glauconite standard GL-O that averaged 24.59$\pm$0.17 (2σ) x 10^{-6} cm^3/g (STP) radiogenic ^{40}Ar for 5 analyses during the study, which is within the recommended standard value of 24.85$\pm$0.48 (2σ) x 10^{-6} cm^3/g [13]. The survey of the procedure included also periodic determinations of atmospheric ^{40}Ar/^{36}Ar loads that averaged 298.7$\pm$1.2 (2σ) relative to the recommended 298.6$\pm$0.4 (2σ; [14]. The age values that have here no connection with a stratigraphic age outline the ^{40}K/radiogenic ^{40}Ar ratio, were calculated using the usual decay constants ([15]. The ^{87}Sr/^{86}Sr ratios of the nontronite clay, the peat fraction and three mixtures were also determined. Approximately 3 to 5 μg of Sr were separated prior to the Sr isotope analysis following a standard ion chromatography procedure on cation-exchange columns with double-distilled 2N HCl as the eluent [16]. After the column separation, about 1 μg of Sr was loaded onto a Ti filament and analyzed for the ^{87}Sr/^{86}Sr ratio on a multi-collector mass spectrometer. To compensate for any isotope fractionation during the isotope measurements, the determined ^{87}Sr/^{86}Sr ratios were normalized to an ^{86}Sr/^{88}Sr ratio of 0.11940. The external reproducibility of the ^{87}Sr/^{86}Sr ratio was controlled by periodic analysis of the NBS987 standard that provided a mean ratio of 0.710227$\pm$0.000017 at the time of the study.

The major and trace elements were analyzed following the procedure of [11]. The international standards BEN-1 and GL-O were included in the analytical procedure together with the selected aliquots to constrain best the analytical accuracy and internal reproducibility.

3. RESULTS AND DISCUSSION

The first set of experiments combined various clay minerals digested by the same type of earthworms following the above-described procedure (Table 1). The selected clay minerals included a K-rich illite and three K-poor smectites (Figure 1A): a hectorite characterized by Na in the interlayer sites and Mg and Li in the octahedral sites, a saponite characterized by Ca and Na in the interlayer sites and Mg and Fe in the octahedral sites, and a nontronite with a Fe-rich octahedral site [17] (Figure 1B). The pH varied from 4.63 at the beginning of the nontronite and peat interaction to 4.24 after 8-week interactions, and from 5.00 at the beginning of the earthworms digesting a mixture of nontronite with peat to 4.92 at the end of the digestion. After digestion, the impact of the earthworms on the clay minerals was checked by X-ray diffraction.

The [001] main peak of the nontronite mixed with peat moss was measured at 15.39 and 9.70° (2θ) after air-drying and heating. The earthworm digestion moved the peaks at 15.09 and 9.80° (2θ), respectively. After clay glycolation, the first peak moved from 15.39 to 17.24° (2θ) with peat and from 15.09 to 17.14° (2θ) with peat after earthworm digestion, the duration of the experiment being the same. The impact of the earthworm digestion is negligible at the interlayer site of the illite (Table 1). The K_2O content increased slightly by 6%, while the so-called age that combines the amounts of K and radiogenic ^{40}Ar, decreased by 2.8%, which is within the analytical uncertainty. In the case of the saponite experiment, the K_2O content decreases by 42% after digestion, and the so-called age increased by 37.8%. As the content of radiogenic ^{40}Ar decreases also, it is plausible that the earthworm digestion impacted the few K-keyed interlayers with a loss of about half of the K_2O and a third of the radiogenic ^{40}Ar. In the case of the hectorite experiment, the earthworm digestion induced an increase of both K_2O and radiogenic ^{40}Ar components of the K-rich interlayers with, again, a higher removal of K than of radiogenic ^{40}Ar, inducing a 37.6% increase of the so-called age. Finally, digestion is the most visible on the nontronite mineral: its K content increased by 121%, while that of the radiogenic ^{40}Ar increased by 105%, which increased the so-called age by 182%. In summary, the hectorite- and nontronite-type smectites reacted similarly when digested by the earthworms, which suggests a different interlayer organization for saponite obviously regulated by Ca and Na instead of Mg and/or Fe.

A further step of the experiment was based on the examination of the elemental variations of nontronite, and moss peat mixtures digested or not by the earthworms (Figure 2; Table 2). After the elapsed 8-weeks interaction of the smectite and peat with the earthworms, the K/Rb ratio of the leachate was of 145 and that of Sr/Ca was of $5.5x10^{-3}$, with a total dissolved amount of 327 μg pro gram of the mixture. The same amount of peat digested by the earthworms during the same duration gave a far higher leached K/Rb ratio of 8430, while that of the Sr/Ca ratio remained at $5.5x10^{-3}$, for a slightly higher total dissolved content of 553 μg/g. The same amount of digested nontronite during the same duration gave a K/Rb ratio of 1000, a Sr/Ca ratio of $5.0x10^{-3}$ and a total dissolved solute content of 976 μg for the leaching solution. The final step combining smectite and peat digested together by the earthworms during 8 weeks provided a K/Rb ratio of 683, a Sr/Ca ratio of $5.5x10^{-3}$ and a significantly higher total dissolved solute content of 1645 μg pro gram of the mixture.

The use of the ^{87}Sr/^{86}Sr ratio for decrypting mixtures of minerals and organics digested by animals remains somehow unexplored. On the basis of a quite constant Sr/Ca ratio for the various mixtures, the purpose was to use this ratio as an independent tracer for each component of the mixtures that are potentially released in the environment by earthworms (Table 3). In the case of a mixture of undigested nontronite and peat, a simple equation with two unknowns provides an amount of 30% of nontronite for 70% of peat for the measured ^{87}Sr/^{86}Sr ratio. After digestion of the mixture, the earthworm contribution is of 45% based on the ^{87}Sr/^{86}Sr ratio of the dejection and of 55% for the nontronite and peat mixture (Figure 2).

In summary, the results show: (1) an analytically constant Sr/Ca of about $5.5x10^{-3}$; (2) a continuous increase of the total dissolved load in the solutes; (3) the highest K/Rb ratio by the triple combination and the lowest by the smectite and earthworm combination, (4) a changing behavior of K from hectorite and nontronite reacting similarly when digested by the earthworms, relative to saponite, which might be due to a different interlayer organization, and (5) the earthworms contribution is of 45% of the solid dejections, that of the peat being of about 40% and that of the nontronite of about 15%, on the basis of the ^{87}Sr/^{86}Sr tracing. A further estimation of the dejected peat and smectite mixture allows some more evaluations based on the contents of the leaches. The total solute release of 1645 ng/g characterizes the three components when combined. It allows a further estimated release of about 670 ng/g by the peat, about 1320 ng/g by the earthworms and about 1090 ng/g by the nontronite (Table 1). Also, and as already stated, the ratio Sr/Ca is very constant for all combinations of the three smectite, peat and earthworms contributions. Apparently, none of the analyzed components releases preferentially any of these two elements, which inclines to state that crystal dissolution is not a major process here. In the case of the K/Rb ratio, the releases are very low for the earthworms at about 540 ng/g, while extremely high at about 7745 ng/g for the nontronite and also very high for the peat at about 7890 ng/g.

The present experiments including the K-Ar and ^{87}Sr/^{86}Sr analyses show that the composition of earthworm dejections depends mostly on the type of digested clay minerals. Illite is clearly not affected, while the various smectites are. In other words, no impact is detectable when the interlayers are keyed by K cations, while it is the case when the interlayers are accessible to the organics of the earthworms' digestive system. For the smectites *stricto sensu*, the impact depends on the internal organization of each mineral: for the Na-Ca saponite, for instance, the earthworms induce a larger removal of K than of radiogenic ^{40}Ar, which is somehow surprising as the first is keyed in the interlayer sites, while the second is only squeezed in them without any connection. One might, therefore, expect that any action on smectite-type interlayers favors a larger release of the radiogenic ^{40}Ar relative to the radioactive K. This is also not the case here for the Mg-Fe hectorite for which the earthworm digestion induces an increase of both the very small amount of K and an even larger increase of radiogenic ^{40}Ar (Figure 3).

In the case of peat moss mixed or not with a smectite and digested by the earthworms, the highest release in the leaches is of 1645 μg/gram. It can be deduced from this amount that peat contributes for about 669 μg/g, which is slightly above the released amount from the sole peat digested by the earthworms and about twice the leached amount of combined smectite and peat. Obviously the smectite and the earthworms did retain part of the peat contribution. If on the other hand, one admits that the earthworms did not contribute but did consume some of the cations, the smectite supplied about 327 μg/g and the peat provided about an amount varying between 553 and 669 μg/g pro gram of treated supply. The use of the ^{87}Sr/^{86}Sr ratio of leachates for decrypting mixtures of mineral and organics digested by animal types is far beyond a basic application. Its values are strictly related to different combinations of the nontronite digestion experiment (Table 3; Figure 2). The impact of the earthworms on the ^{87}Sr/^{86}Sr ratio of the nontronite is closely that of the peat. Interestingly, the content of radiogenic ^{40}Ar before and after the different digestion experiments outlines very different behaviors of the digested smectites. If saponite loses only a small amount of radiogenic ^{40}Ar during the digestion process, the two other smectites, namely hectorite and nontronite, adsorb significant amounts of radiogenic ^{40}Ar during the digestion by the earthworms. These results outline different behaviors for the interlayers of the smectites depending on their type, while that of illite is apparently not affected at all.

The Sr/Ca ratio of the various mixtures being somehow constant, the purpose was to decrypt the Sr isotopic ratio of the various leaches for the contribution of each component relative to the earthworm action. On the basis of the nontronite and peat mixture, the Sr isotopic ratio allows an approximation of 30% for the nontronite contribution and of 70% for the peat of the nutrients, while the digestion by the earthworms modifies their contribution to 45% for the worm action and 55% for the digested nontronite and peat mixture. In summary, the data obtained by the various experiments suggest that the smectite component, whatever its type, supplies about half of the detritus. For this mineral supply, the interactions depend strictly on the type of minerals subjected to digestion. It also looks like the earthworms digest varied amounts of peat depending on what they extract from associated clay material.

4. CONCLUSIONS

Earthworm digestion affects variably the interlayer spaces of the digested smectites, while not affecting at all those of the illite and therefore, by extension, those of micas. The digestion extracts variable elemental amounts from nutrients depending on the type of clay material and more precisely on the type of the interlayers of the smectite-type material. Cation exchange is not necessarily a dominant mode along which clay minerals are modified by an earthworm digestion. K-lacking interlayers are more cleaned than K-keyed interlayers. Mineral dissolution seems not to be a major removal process that is rather dominated by exchanges in the interlayers depending on the strength of the elemental connections.

Two analytical applications deserve a further comment about the unusual K-Ar dating and Sr isotopic tracing to unravel the contribution of mineral and organic components digested by earthworms. The K-Ar dating method shows that the behavior of K and radiogenic ^{40}Ar depends on the type of digested smectites and not on the impact of the digestion process. The most spectacular impact was found on the Fe-rich nontronite. The Sr isotopic ratio of the leachates from a nontronite and peat mixture, before and after digestion, suggests that nontronite contributes for 30% and peat for 70% of the releases into the nature, and that the earthworm action represents close to half of the contribution of these digested materials, the other half being due to the digested components themselves.

A plausible explanation could be that organic compounds derived from bacterial activities in the intestine of the earthworms become sequestered in the interlayer sites of clay minerals where ion exchanges are expectedly hindered significantly. The plant materials represented here by the peat moss are a substantial source of the solutes, therefore potentially transferred in surface waters of soils.

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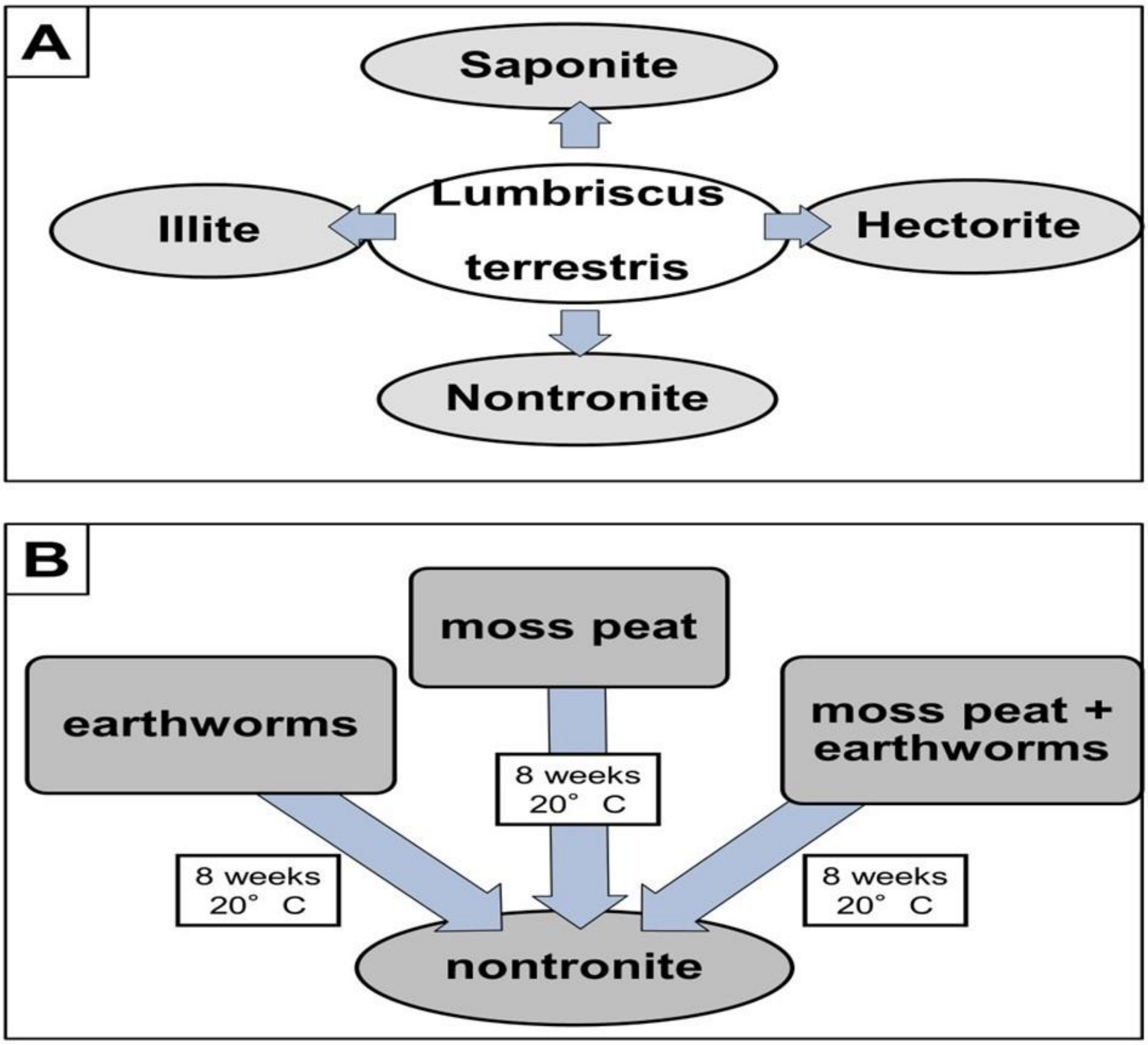

Figure 1: XRD diagrams of nontronite before and after worm digestion, as well as of a mixture of nontronite and peat, again before and after digestion.

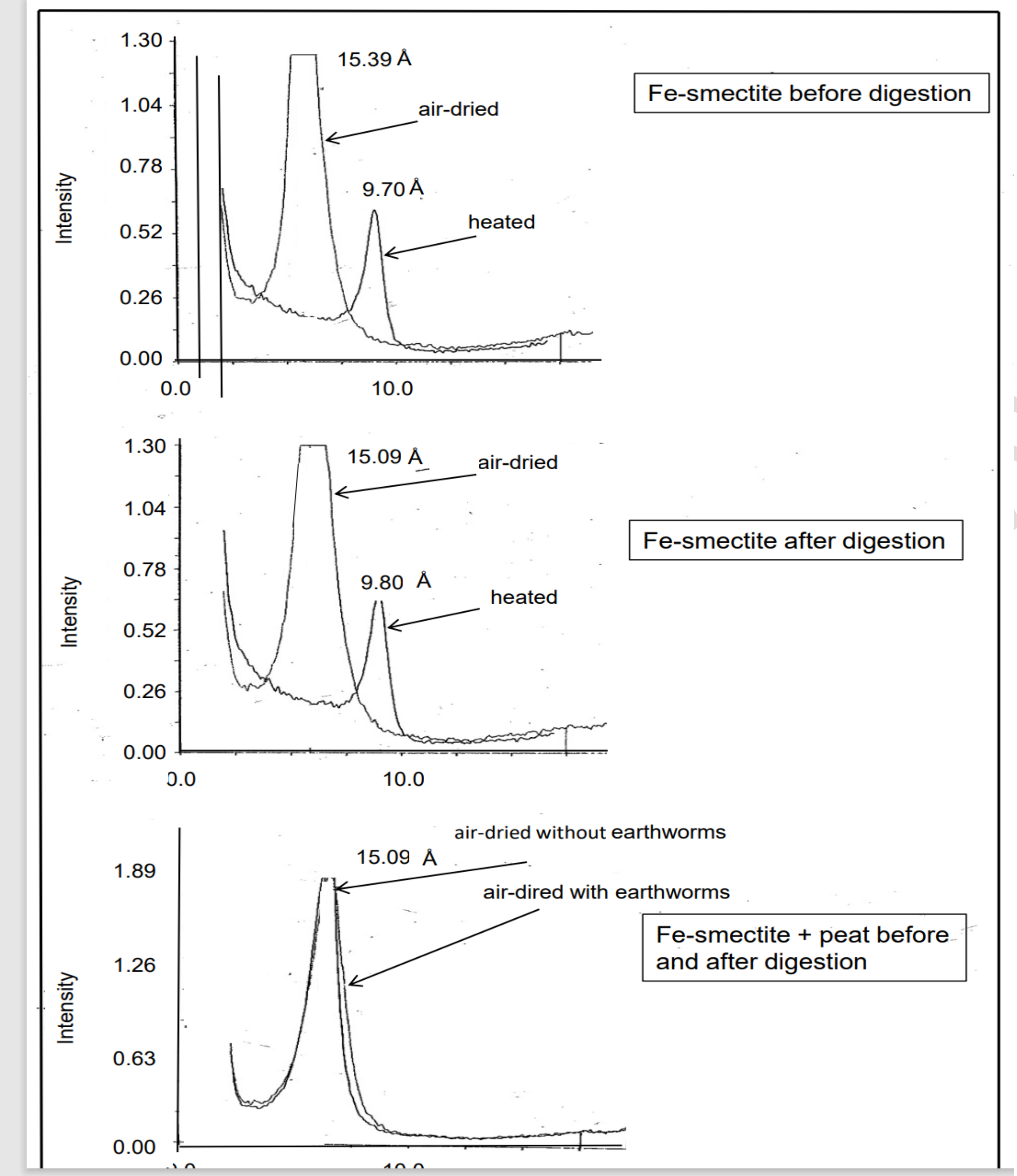

Figure 2: ^{87}Sr/^{86}Sr data of the various components from long-term earthworm digestion experiments.

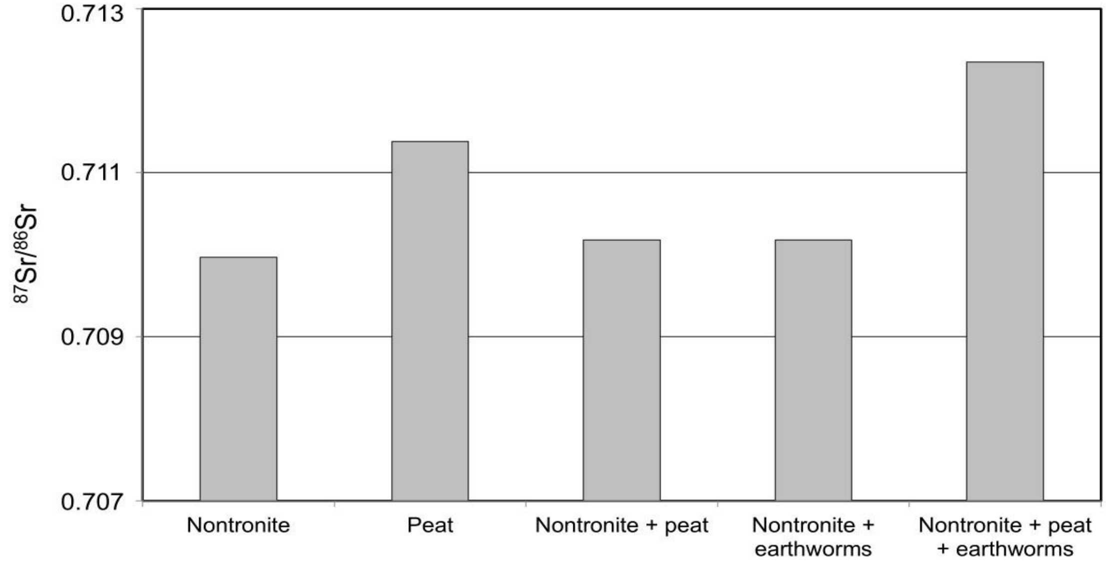

Figure 3: Radiogenic ^{40}Ar contents of the illite and the three smectites before and after earthworm digestion.

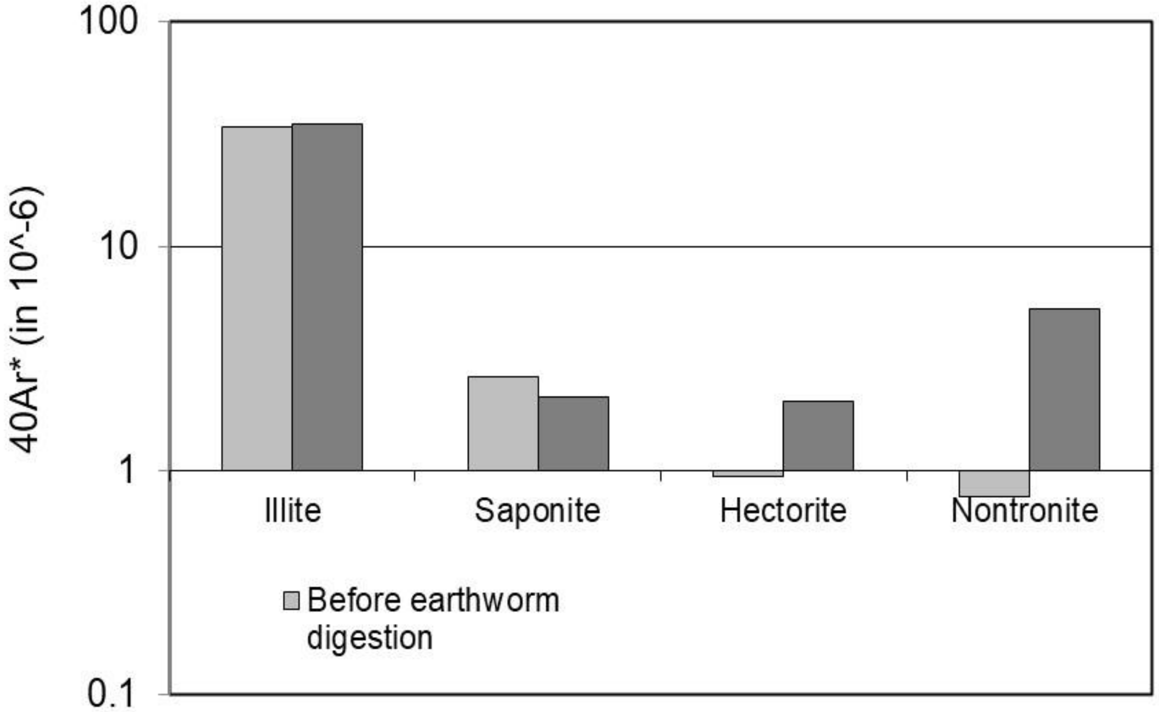

Figure 4. Earthworm digestion process

TABLE 1: K-Ar data of the illite and the three smectites separates before and after earthworm digestion.

Illite	
before earthworm digestion	after earthworm digestion
K_2O = 2.18%	K_2O = 2.31% (+6.0%)
$^{40}Ar^*$ (cc/g) = 33.98×10^{-6}	$^{40}Ar^*$ (cc/g) = 34.81×10^{-6} (+2.4%)
Ar* = 97.2%	Ar* = 97.1% (-0.5%)
‘age’ = 428±11 Ma	‘age’ = 416±10 Ma (-2.8%)
Saponite	
before earthworm digestion	after earthworm digestion
K_2O = 0.50%	K_2O = 0.29% (-42.0%)
$^{40}Ar^*$ (cc/g) = 2.63×10^{-6}	$^{40}Ar^*$ (cc/g) = 2.13×10^{-6} (-19.0%)
Ar* = 81.3%	Ar* = 48.4% (-40.5%)
‘age’ = 156±5 Ma	‘age’ = 215±10 Ma (+37,8%)
Hectorite	

before earthworm digestion	after earthworm digestion
K_2O = 0.06%	K_2O = 0.09% (+50%)
$^{40}Ar^*$ (cc/g) = 0.94×10^{-6}	$^{40}Ar^*$ (cc/g) = 2.04×10^{-6} (+117.0%)
Ar^* = 49.7%	Ar^* = 73.2% (+47.3%)
'age' = 431±22 Ma	'age' = 593±29 Ma (+37.6%)
Nontronite	
before earthworm digestion	after earthworm digestion
K_2O = 0.14 %	K_2O = = 0.31% (+121%)
$^{40}Ar^*$ (cc/g) = 0.77×10^{-6}	$^{40}Ar^*$ (cc/g) = 5.28×10^{-6} (+585.7%)
Ar^* = 4.29%	Ar^* = 21.68% (+405.4%)
'age' = 164±46 Ma	'age' = 463±43 Ma (+182%)

TABLE 2: Comparisons of elemental contents of various mixings of clay minerals, peat moss, digested or not by the earthworms.

nontronite + peat
Total dissolved solute content = 327 µg/g clay K/Rb = 145 U = 0.6 ng/g clay Th = 3.0 ng/g clay U/Th = 0.20 Sr/Ca = 5.5×10^{-3}
peat + earthworms
Total dissolved solute content = 553 µg/g clay K/Rb = 8430 U = 3.5 ng/g clay, Th = 4.9 ng/g clay, U/Th = 0.71 Sr/Ca = 5.5×10^{-3}

nontronite + earthworms
Total dissolved solute content = 976 μg/g clay K/Rb = 1000 U = 1.7 ng/g clay, Th = 3.7 ng/g clay, U/Th = 0.46 Sr/Ca = 5.0×10^{-3}
nontronite + peat + earthworms
Total dissolved solute content = 1,645 μg/g clay K/Rb = 683 U = 1.9 ng/g clay, Th = 11.6 ng/g clay, U/Th = 0.16 Sr/Ca = 5.5×10^{-3}

Table 3: $^{87}Sr/^{86}Sr$ isotopic ratios of nontronite, peat, nontronite and peat, nontronite digested by the earthworms and all three combinations together.

Components and assemblages	$^{87}Sr/^{86}Sr$
nontronite	0.70997
peat	0.71138
nontronite + peat	0.71018
nontronite + earthworms	0.71029
nontronite + peat + earthworms	0.71235