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Effects of long-term tea (*Camellia sinensis*) cultivation on the earthworm populations in northern Iran

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Abstract

The earthworms' abundance is usually one of the main indicators of healthy and productive soils. However, agricultural management practices affect the earthworm population and activities. Although there is a lot of information that shows the relation between land use/land cover change and earthworms activity, very little is known about these effects under tea cultivation. Thus the current study was done to determine the effects of long-term tea cultivation on the earthworm's population and abundance in the tea plantations of Iran to distinguish effects of these practices on soil properties concerning earthworms. Hence, 58 locations of tea cultivations were randomly selected in Guilan and Mazandaran province. Earthworm were sampled by manually excavating and sorting four 30×30 cm pits by 30 cm deep in each location. Earthworms were enumerated in the field and taken to the lab for identification. Once identified, the earthworms will dry in the oven at 60°C for 48h and the dry weight registers. Some physicochemical properties of the mineral soils were determined in the laboratory. The finding indicated that the earthworms were only observed in the two from 58 locations: Bazkiagorab (Lahijan) and Shekarposhteh (Tonekabon). Three species as *Perelia kaznakovi*, *Aporrectodea trapezoides*, and *Dendrobaena veneta* were recorded from Bazkiagorab but only *P. kaznakovi* was identified in Shekarposhteh. The total population of all identified earthworms was 22 and 3 m^{-2} in the Bazkiagorab and Shekarposhteh, respectively. Results of the physicochemical analysis showed that 35% and 51% of the soils had a pH less than4.5 and organic carbon less than2%, respectively. Available phosphorus and potassium in 80% and 65% of the soils were less than 25 and 150 mg/kg, respectively. It can be concluded that monoculture and long-term tea cultivations had a negative effect on the earthworm population, in addition, it has strongly acidified the soil. It is recommended that native nitrogen-fixing trees mixed planted with tea, and more attention should be paid to nutrient Best Management Practices in tea plantations.

Keywords: Iran, acid soil, *Perelia kaznakovi*, *Aporrectodea trapezoides*, *Dendrobaena veneta*.

Introduction

Soil earthworms are one of the most important soil macrofaunae that lead to the transformation of materials into another physical stage. Soil earthworms, by eating up the particles of plant and soil residues, are a part of food chain affect important soil quality indicators such as the structure and dynamics of organic matter (Pulleman, 2002). They contribute to decaying organic matter and to incorporate them into soil. Although their drilling activities improve soil structure and increase soil aeration (Edwards and Lofty, 1972).

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The abundance of earthworms is related to the characteristics of soil and climate. Earthworms are usually absent at very acidic pH (less than 3.5) and they rarely found at pH less than 4.5 (Curry, 1998). The low number of earthworms in acidic soils has been linked to a lack of Ca^{2+} ions in acidic soils, trees and shrubs produce unpalatable and low quality foods and a C/N ratio>60, which is undesirable for earthworms. The quality of plant residual has a greater impact on the population of earthworms than the abundance and stock of soil organic matter (Boström and Lofs-Holmin, 1986). In addition to the availability of nutrients, the quality of leaf litters is affected by the content of carbohydrates, the concentration of phenolic compounds (especially tannins), etc. (Graff and Satchell, 1967).

Camellia sinensis (L.) Kuntze has been imported to Iran for more than 100 years. Nowadays, the area of plantation may reach to over 25000 ha (ITO, 2018). In recent years, excessive use of nitrogenous fertilizers, leaching of nutrients from the soil, and failure to use soil modifiers such as lime and dolomite have reduced the pH of soils in tea gardens (Shirinfekr, 2018). Jamatia and Chaudhary (2017a,b) reported that out of 17 species of earthworms observed, 13 and 15 species of earthworms were recorded in managed tea Cultivations and destroyed tea cultivations, respectively. Density (56 m^{-1}) and biomass (27 g m^{-1}) of non-native species were significantly higher in the managed tea gardens than the destroyed ones (23 m^{-1}, 17 g m^{-1}). Luthfiyah (2014) reported that the diversity of earthworms in the three tea gardens of research stations is low where *Pontoscolex* sp. had the highest density. Correlation between the density of earthworms and physical and chemical properties was observed in tea fields of PTPN XII region. Soil moisture and pH have a positive effect on the population and density of earthworms. Jamatia and Chaudhuri (2017a,b) identified 15 species of earthworms from four families by study on the structure of earthworm community in the tea Cultivations of four Tripura regions of India. Overall, the average density and biomass of earthworms, regardless of their age group, was 212 m^{-1} and 51.7 g m^{-1}, respectively.

Due to the sustainable agriculture approach and the use of biological materials in tea plantations, the population and abundance of earthworms in these climatic conditions should be investigated to determine their potential for improving the physicochemical and biological properties of the soil. This aim might be achievable by rearing and releasing of the most suitable species in the tea plantations of north Iran as there is no similar report. So far in Iran, the effect of tea cultivation on earthworm abundance as one of the most important indicators of soil quality has not been reported. This study was therefore aimed at determining the population of earthworms., identify the species resistant to acidic soil, and their relationship with soil characteristics under long term tea plantation in north of Iran.

Material and Methods

Study Sites

This study was conducted in Iranian tea cultivations in the north of Iran. This area is located in Guilan and Mazandaran provinces, between the latitudes 37°12’39’’ to 37°50’15’’ N and longitudes 49°55’57’’ to 50°45’31’’ E. The minimum and maximum altitudes of the study area are -6 m and 485 m above sea level respectively. Mean annual precipitation and temperature are 1186 mm and 17.5°C, respectively.

More than 100-y ago, these areas were covered by Hyrcanian forests trees. Later on, that tea plants (*Camellia sinensis*) imported from India and introduced in two provinces, these forests were clearly cut and changed to the tea plantations. Seven study locations in different conditions were selected as Table 1. Fiftyeight sites (Table 1) were randomly selected and their coordinate were recorded using GPS (Figure 1).

Table 1. Distribution the number of samples in different locations

Region	Visited site	Sampled sites
Astaneh Ashrafieh	11	2
Lahijan	34	7
Langrood	15	6
Roodsar	53	28
Amlash	35	10
Tonekabon	7	2
Fooman	6	3
Total	161	58

Methods

At each study site the earthworm density, earthworm biomass, earthworm species, earthworm ecological group and soil parameters were measured. On each site 1 kg of soil samples for chemical analysis were collected from four points placed at the apices of a 10-m side square. Soil samples were air-dried, ground through a 2 mm sieve and analyzed for soil pH (1:1 w/v) in a soil: water suspension, organic carbon using Walkley-Black method, available P content by Bray-1 method, and available potassium by 1N NH_4OAc method (Motsara and Roy, 2008).

Figure 1. Locations of soils sampled

Earthworms were collected in spring of three years ago using four random samples (30cm×30cm×30cm) with 15 m distance between the pits. By removing the litter layer, the soil was gradually dug to a depth of 0-30 cm using a shovel. The soil from each plot was spread on polyethylene sheets and hand-sorted (Thielemann, 1986). The earthworms were enumerated, collected and were brought into the glass jars with sufficient amount of moist soil to the laboratory.

In the laboratory, the collected earthworms were washed, weighed, and sexual maturity and body color were recorded. The earthworms were fixed with 70% alcohol and morphologically identified according to the available keys (Edwards and Lofty 1972; Latif et al., 2009; Reynolds and Mısırlıoğlu, 2018) using a stereomicroscope.

Pearson's correlation analysis was performed between various physico-chemical parameters of soil and earthworm density. All the analyses are done with the help of SPSS 18 software program (SPSS, 2018).

Results

Earthworm density, biomass and ecological group

The earthworms were observed only in the two locations of 58 location at Lahijan and Tonekabon counties (Table 2). These specimens were identified as *Perelia kaznakovi, Aporrectodea trapezoides*, and *Dendrobaena veneta* based on key morphological characteristics such as body length and diameter, length, and position of Clitellum (Table 3). Three species were collected in Bazkiagorab (Lahijan) while only one species observed in Shekarposhteh (Tonekabon). Mean biomass of the earthworms varied from 0.65-1.38 g m^{-1} and 0-1.5 g m^{-1} in Bazkiagorab and Shekarposhteh, respectively. The population of earthworms in the Tonekabon region was 3 m^{-1} while it was determined 22 m^{-1} in Lahijan region (Table 3).

Table 2. Abundance and biomass of collected earthworm

Species	Lahijan		Tonekabon	
	Density ind.m^{-2}	Biomass gm^{-2}	Density ind.m^{-2}	Biomass gm^{-2}
Perelia kaznakovi	9	1.38	0	0
Aporrectodea trapezoides	5	1.26	3	1.5
Dendrobaena veneta	8	0.65	0	0
UTM(X,Y)	4120199	406786	4074973	477472

Table 3. Morphological characteristics of collected earthworm

Species	Ecological group	Body Length(mm)	Diameter(mm)	Clitellum position
Perelia kaznakovi	Endogeic	55-68	5-6	27-35
Aporrectodea trapezoides	Endogeic	80-140	3-6	26-35
Dendrobaena veneta	Epigeic	50-155	4-8	27-33

Perelia kaznakovi

Aporrectodea trapezoides

Dendrobaena veneta

Soil Physico-chemical properties

Descriptive statistics of the physical and chemical properties measured in soil samples are presented in Table 4. The results indicated that 34% of the soils have the pH less than 4.5 and the pH of 12% of the sampled soils was more than 6.0, which is not suitable for optimal tea growth which needs to be reclaimed (Figure 2). In the soils sampled from Tonekabon and Fooman, the pH is more than 5, and in other soils of the dominant pH is less than 5.0.

Table 4. Descriptive statistics of measured soil parameters

Characteristics	pH	OC %	P, mgkg^{-1}	K, mgkg^{-1}	Sand, %	Clay, %
mean	4.78	%	23.70	147	23	51
median	4.75	1.95	5.4	122	23	49
Mode	4.19	2.20	62	67	23	47
Min.	3.40	0.78	0	14	6	22
Max.	6.10	3.67	268	692	50	81
Sd	0.61	0.66	49.57	112	9.35	12.98
variance	0.37	0.43	2414	12628	87	168
Range	2.70	2.89	268	678	44	59
No.	58	58	58	58	58	58

Also, 52% of the studied soils have less than 2% organic carbon, which indicates the depletion of soil carbon, the non-consumption of organic fertilizers, and no return of plant residues to the soil. Organic carbon was appropriate just in 9% of soils (Figure 2). It was determined that 69% of soils have severe deficiencies due to the amount of available phosphorus so it may be inferred a high response of the tea plants to phosphate fertilization. On average, phosphorus deficiency is observed in 10% of soils although almost 17% of the sampled soils required a little phosphate fertilizer application (Figure 3).

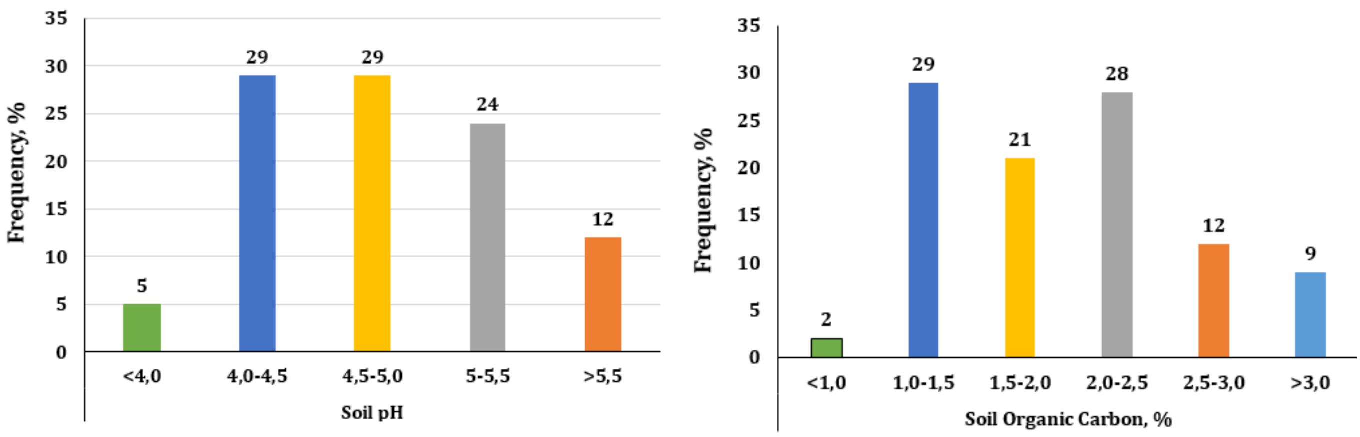

Figure 2. Distribution of soil pH and SOC in the studied soil

Almost, 65% of the studied soils had less than 150 mg K kg^{-1}, indicating a severe potassium deficiency and depletion. In 19% of soils the response of tea plants may be positive to potassium fertilizers, and in 16% of the soils, there is no need to use potassium fertilizers (Figure 3).

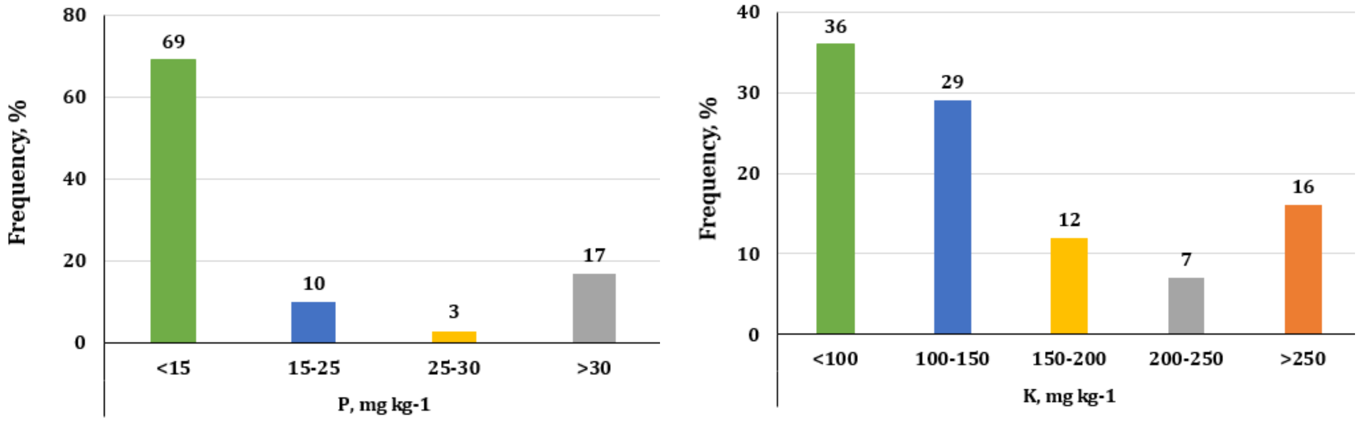

Figure 3. Distribution of available P and K in the studied soil

The texture of more than 50% of the studied soils is suitable for tea cultivation. The amount of clay in 22% of soils was more than 33%, which can reduce the growth of tea by creating unfavorable conditions, and the need for corrective operations such as embedding drainage system and lightening soil texture was necessary (Figure 4).

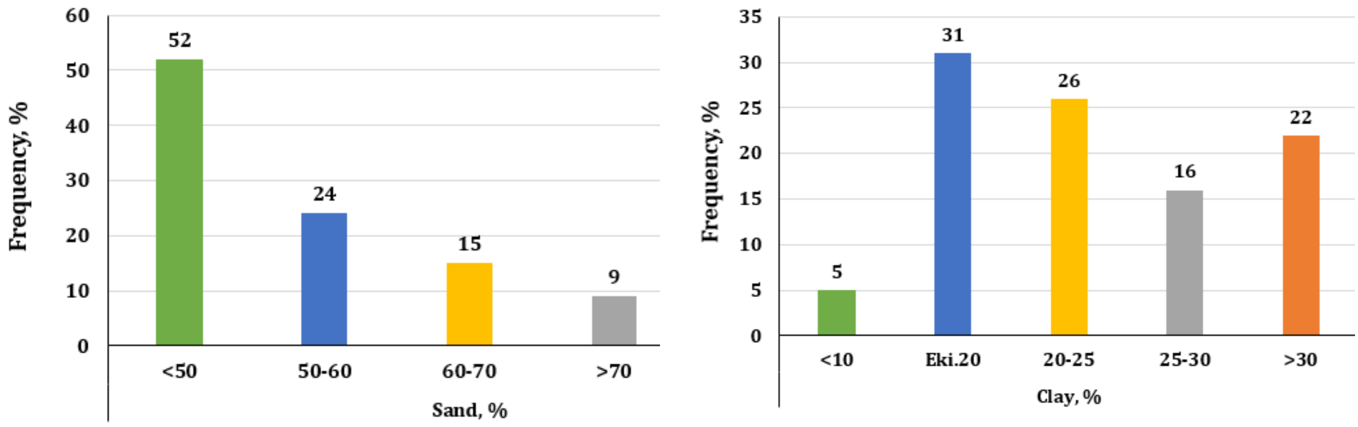

Figure 4. Distribution of soil sand content in the studied soil

Discussion

Earthworm's abundance

Earthworms was absent in majority of the present studied soils. A maximum of three species was observed in an area, indicating severe ecosystem degradation and adverse conditions for growth and survival of the earthworms in Iranian tea plantations. Earthworms are one of the biological indicators to evaluate soil quality and unbalanced physical and chemical properties of soils, it is necessary to improve soil conditions. The number of earthworm's species in each soil is the simplest way to measure species diversity, which usually varies between 4-14 species (Edwards and Bohlen, 1996). Latif et al. (2009) reported *D. veneta* and *P. kaznakovi* species from the central Alborz Mountains in Haraz and the Chalous River (Karaj to Chalous).

According to Radaei and Izadi (2016), the maximum and minimum population of earthworm at the forest of northern Iran, found in alder and spruce stands. Sinha et al. (2010), reported two ecological groups of earthworms in the soils of tea ecosystems. A single ecological system with the same ecological status in the tea garden has fewer ecological earthworms than the forest ecosystem with different ecological niches.

Jamatia and Chaudhuri (2017) reported that endogenic species were generally predominant in the tea ecosystems. In their study, 12 out of 15 observed species were endogenous, similar to our study. According to Harbowy et al. (2017), the absence of epigeic species and a small number of endogenic species (3 out of 15 species) in the tea soil may be attributed to their low plant diversity and high polyphenolic residues.

Tea leaf residues are not popular with earthworms due to their high polyphenol content (Harbowy et al., 2017). According to Edward and Bohlen (1996), the amount of leaf polyphenols had a negative relationship with leaf utilization by earthworm. Jamatia and Chaudhari (2017a,b) reported a positive and significant correlation between earthworm density with pH and organic carbon of soil.

Chaudhuri and Nath (2011) found a correlation between density and biomass of earthworm with soluble sugars, polyphenols, flavonoids and lignin in the leaf residues of rubber cultivation.

Soil moisture and temperature in tea Cultivations have a significance positive and negative correlations with earthworm density and biomass, respectively. Because the body of earthworms is soft, they need moist conditions to survival, maintenance of hydrostatic pressure and prevention from drying out (Najar and Khan 2014). Because, soil compaction was evident and tillage operations were not carried out for a long time, the slow rate of water infiltration, irregular distribution of rainfall and increasing hot days of the year (Kahneh et al. 2019) create unfavorable conditions led to decrease the population of earthworms in Iranian tea gardens.

Since calcium is essential for the growth and the reproduction of earthworms. Deficiency and leaching of this element in the tea soil and excessive consumption of nitrogen fertilizers are among the factors affecting elimination of earthworms.

Therefore, it seems that tea cultivation has reduced the number of earthworms that feed on carcasses and reduced the variety of worms in tea cultivations. However, the presence of some native species in the soil of tea cultivations indicates presence the resistant native species that can be active with foreign species.

Change in Soil Properties

In the current study, 34% of sampled soils had acidic to very acidic pH. also Chien et al. (2019) reported that out of 12 tea gardens tested in Vietnam, four soils have pH less than 3.5 and six have pH = 3.5 (H_2O). Karak et al. (2015) observed that the pH of surface soil in the study areas of Indian tea gardens varied from 3.61-6.81. According to Zhu et al. (2013), the secretion of organic acids from the roots of the tea plant can acidify the soil pH gradually decreased with increasing age of tea plants. Therefore, one of the most important factors in reducing pH of the studied soils can be: i. Excessive consumption of nitrogen fertilizers (urea) in tea gardens. İi. The age of tea plants in most gardens and iii. leaching of nutrients, especially calcium and magnesium from the soil.

The organic carbon of the studied soils showed the ranges between 0.78% and 3.67%. Karak et al. (2017), also reported low levels of organic soil carbon in the tea Cultivations of India and Rwanda. Karak et al. (2015), concluded that low levels of organic carbon in tea soil can be due to low plant residues and high yields of young shoots.

In our study, almost 69% of the sampled soils contained very little or high amount of phosphorus. Chien et al. (2019), reported that the available soil phosphorus-Brya2 in some Vietnamese tea was 54-1107 mg kg^{-1} with an average of 535 mg kg^{-1}. Isobe et al. (2017), reported that the available phosphorus- Brya2 varied from 0 to 260 mg per 100 g in the tea cultivation in highlands in Japan, but most soils have less than 25 mg per 100 g of soil available phosphorus.

The amount of available potassium was less than 150 mgkg^{-1} in 65% of the sampled soils. Ruan et al. (1999, 2013) declared the availability of potassium less than 100 mg kg^{-1} in the soils of Chinese tea plantations. Accordingly, out of 3396 samples of soil tested, 2513 samples had less than 100 mg kg^{-1} of potassium and the average potassium in these soils was 80 mg kg^{-1}.

Moreover, 45% of the soils had 10-20 clay and only 3% of the soils had more than 30% clay. Chien et al. (2019), also reported a soil clay content of 10-32% in Vietnamese tea. They considered the amount of clay to be 18% for the grouping of tea soils and concluded that if leaf quality depends on the growth, yield and yield components of the tea plant, soil texture and related characteristics could be one of the important factors. Therefore, it can be concluded that tea cultivation has reduced the number and abundance of earthworms in Iranian tea Cultivations. However, the presence of some native species in the tea Cultivations indicates that there is a resistant native species in these soils that can be used with foreign species for further research. Also mixed plantation of N_2-fixing tree with tea and nutrient Best Management Practices is recommended in tea plantations

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