

UNIVERSIDAD POLITECNICA DE VALENCIA

ESCUELA POLITECNICA SUPERIOR DE GANDIA

Licenciado en Ciencias Ambientales



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POLITECNICA
DE VALENCIA**



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SUPERIOR DE GANDIA**

**“Soil acidification with a specific
view to aluminium”**

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1-INTRODUCTION

1.1- Soil importance in the ecosystems development.¡Error! Marcador no definido.¡Error! Marcador no definido.

Plant production in natural and semi-natural plant communities is controlled by a number of relationships between biological processes on the one hand and physical and chemical factors of the atmosphere and soils on the other hand. The importance of relationships within an ecosystem consists in the fact that any change in the system component no matter how small can induce changes in its other part. Therefore the soil plays an important role within forest ecosystems because it affects resistance and resilience to a considerable extent and thus the stability of the whole system (klimo *et al.*, 2006).

Attention is paid both to the development of soil (soil processes and soil properties) and to relationships between soil and plant (soil fertility). The rate and intensity of soil processes have already been the subject of interest of soil scientists for a long time because the rate and intensity not only explain potential anthropic impacts on these processes in the past but also they are a condition for elaborating the prediction of potential consequences of human activities in the development of soil properties closely related to forest soil fertility. The soil itself can be regarded as a relatively stable component of forest ecosystems considering the long-term character of its genesis. Of course, possible destructions of this subsystem can result in a serious reduction of the production level of forest stands. Therefore potential consequences for changes and development of soil processes and soil properties are important from the aspect of present evaluation as well as from the aspect of their future development (klimo *et al.*, 2006).

1.2- Acidification

Acidification may begin as a result of a combination of factors including among them: high rainfall (dilution and leaching of base cations), atmospheric deposition

mainly (NO_x , SO_x , H_2SO_4 , HNO_3), poor management of coniferous forest, granite parent rock

and other basic soil forming factors. All this may produce an increase in H^+ and therefore an acid composition in soil.

In the Czech Republic presents a serious problem in forest soils of mountainous areas. Acidification is the process that leads to depletion of base cations, pH decrease, development of lower quality humus and is controlled by a number of factors, like acid deposition, forest type, parent rock, altitude, etc. (Boruvka *et al.*, 2005).

There is a decrease in the availability of nutrients (P, Mg, Ca) in places where they are often absorbed by plants having been exchanged for other cations such as H^+ or Al^{3+} . The aluminium will produce a decline in growth in length of the plants and will do acting at two levels: by inhibiting cell growth and inhibiting cell division.

1.2.1- Natural causes

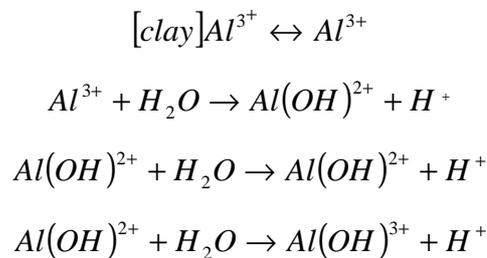
Soil acidification could happen natural causes such as high rainfall that promotes the dilution and leaching of base cations to the aquifer or vegetation growth that leads to exhaustion.

Initial soil vulnerability to acidification is conditioned naturally by parent rocks and other basic soil forming factors. In general, mafic rocks such as basalts or gabbros have greater weathering potential than felsic rocks such as granite due to the lower stability of their constituent's minerals and their greater concentrations of base cations.

The processes involved in soil formation are the same in all climates, however, in tropical regions humid weathering rates are higher than erosion rates, which allows the formation of highly weathered profiles (Bohn *et al.*, 1985). When young, the soil has a pH close to neutral, however, over time, the contents of potassium (K), calcium (Ca), magnesium (Mg) and sodium (Na) are reduced and the soil becomes achieve some degree of acidity (Brady, 1984). The process of acidification of soil takes hundreds or thousands of years to get even and steady state is the result of several mechanisms.

One such mechanism is generated by the rain water, by moving the lower layers of soil, it carries with it quantities of Ca, K, Mg and Na have been shed from the surface of clays and other colloids. The spaces left by the cations removed by water are then occupied by H⁺ than to increase in concentration in the soil, it begins to impart acidic properties. Therefore, the degree of acidity that develops in a soil depends on the parent material and the speed and amount of rainfall.

The second mechanism responsible for soil acidification is the process of hydrolysis of aluminium (Al) (Bohn et al., 1985). The process starts with the trivalent aluminium ion (Al³⁺) to that he will neutralize their positive charges through the hydrolysis of water (H₂O ↔ H⁺ + OH⁻). During this process, the ion Al³⁺, once removed from the surface of the clay reacts with water in the soil resulting from the hydrated compounds and ions free H⁺ than to be released into the soil solution increase the acidity. The presence in solution of different types of ions of Al is determined by soil pH. The Al³⁺ ion which is represented only at pH values below 4.7, the Al(OH)₂⁺ between 4.7 and 6.5, and Al(OH)₃ between 6.5 and 8.0. In simple terms the process is as follows:



A third mechanism that influences the acidification of soils is the root activity, namely the absorption of nutrients. All plants without exception, conifers or not, native or exotic species, agricultural or forestry land extracted base cations (Ca²⁺, K⁺, Mg²⁺) for use in biomass production. During the process of absorption of these nutrients (interphase root / soil) the charge balance inside the root should be maintained, so that when absorbed Ca²⁺, K⁺ and / or Mg²⁺, is released to soil solution H⁺ to restore , inside the root, the power balance (Bohn et al., 1985). The H⁺ released enter the soil solution by increasing its concentration and increasing acidity.

A fourth mechanism responsible for soil acidification is nitrification. Since the feed on plants only chemical elements in soil are in ionic form, the complex organic compounds rich in nitrogen (N) should be altered to reach its chemical expression simplest form mineral. The conversion of ammonium results H^+ which contributes to soil acidification.

The fifth mechanism responsible for soil acidification is driven by the accumulation of organic matter on mineral soil surface. Once deposited, the organic matter breaks down to form organic acids (COOH) that moving into the soil release H^+ . This H^+ replaces the base cations (Ca^{2+} , K^+ and Mg^{2+}) on the surfaces of clays.

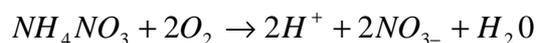
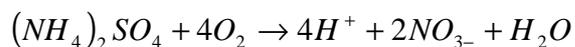
1.2.2- Anthropogenic causes

Anthropogenic effects on soil acidification include mainly acid deposition, forest management, liming, use of nitrogen fertilizers etc. Level of atmospheric deposition depends on the proximity of emission sources, wind direction, and terrain.

Acid rain makes the soil acidic species such as NH_4^+ and Fe^{3+} but especially nitric and sulfuric acids produced by oxidation in the atmosphere of sulfur oxides and nitrogen. Though sulphur emissions decreased dramatically in last decades, the effect of S accumulation is long-term, moreover, the nitrogen deposition is still fairly high (Boruvka *et al.*, 2005). The pollutants include elements that are required normally by the plant physiologically. The increased flux of these elements/compounds in the atmosphere, usually due to human activities, often results in the build up of high concentrations in the soil and plants over and above the normal levels living organisms can cope with. These materials are transported mainly in form of atmospheric dust (Adetunji *et al.*, 2001).

The use of nitrogen fertilizers can increase soil acidity due to nitrification in the process of releasing H^+ . The degree of acidity depends on the nitrogen compound used. The most commonly used are urea, ammonium nitrate and ammonium sulfate.

During the reaction on the ground the three compounds give the same amount of N but releases various amounts of H⁺:



These reactions are the most acidifying effect of ammonium sulfate for ammonium nitrate and urea. However, one can not generalize about this effect in all soils, usually the buffering capacity of clay soils makes the acidity induced by these three sources is lower, particularly in the case of ammonium sulphate (*Efecto de diferentes fuentes de N amoniacal sobre la acidificación del suelo. Reporte de International Fertilizer Development Center (IFDC). Agosto del 2001*).

1.2.3- Acid stress

Acid stress is defined as the input of hydrogen ions into the top-soil. Acid stress can result from acidic deposition of air pollutants, from biomass utilization, and from the natural biological activity of ecosystems. Any one of these sources can dominate the stream of protons entering the soil. The acid stress due to air pollution can result from the direct deposition of hydrogen ions or from the indirect effect of acid-producing substances such as the dry deposition of SO₂ (Kauppi *et al.*, 1986).

1.2.4- Aluminium stress

Aluminium mainly affects plants by inhibiting radical growth. This can be seen in the primary and lateral root apices, which also become thick and turn brownish-gray (Rout *et al.*, 2001). These symptoms become evident after a few minutes or hours of the plants being exposed to micromolar concentrations of Al in hydroponic solutions (Rengel and Zhang, 2003). Radical inhibition coincides with a decline in cell division (Frantzios *et al.*, 2001) and elongation of the root cells, which then induces significant

rigidification of the cell wall by crossing with pectins (Jones *et al.*, 2006). This alteration prevents the water absorption essential to the transport of nutrients (Zheng and Yang, 2005). Excesses of Aluminium may also induce symptoms of a Fe deficiency.

Several studies indicate that Al affects the normal operation of cell membranes, causing enzymatic disorders and affecting the nuclear DNA (Maustakas *et al.*, 1992). In addition, Aluminium is closely linked to other DNA-associated molecules, such as phosphorylated proteins (histones) (Kochian, 1995), and may interfere in the normal operation of the Golgi apparatus and in the peripheral cells of the apex of intact roots (Rout *et al.*, 2001). All This would affect the direction of the microtubules, which is closely related to cell expansion (Zheng and Yang, 2005).

1.3 Biological indicators and soil chemical criteria.

In the case of critical loads of acidity to forest soils, the biological indicator organism is the tree and the chemical criterion generally used is the molar ratio of base cations to aluminium concentrations in the soil solution of the rooting zone. In principle, if this ratio is persistently low ($(Bc/Al) < 1.0$), Al toxicity to the tree roots will ensue, with a corresponding decline in growth and performance of the trees.

Cronan and Grigal (1995) reviewed an extensive set of studies concerning Ca-Al interactions and Ca/Al ratios and recommended application of the Ca/Al ratio indicator for assessment and monitoring of forest health risks in regions where the soil base saturation is less than 15% of effective cation exchange capacity. Based on 36 data sets reported in literature, they estimated that there is a 50:50 risk of adverse impacts on trees when the soil solution Ca/Al molar ratio is 1.0_0.5 or below (Cronan and Grigal, 1995). The study has some uncertainties associated since the results are based on seedling responses under controlled conditions as well as a mixture of more and less sensitive species studied under varying treatment conditions (Holmberg *et al.*, 2001).

1.4 Impacts

The most significant impact caused by acidification of the environment is acidic leaching from soil to surface water and groundwater. The water draining acidified soil contains high concentrations of aluminium (Van Bremen and Verstraten, 1991). This element produces significant negative impacts on surface water (impairment of aquatic life) and groundwater (contamination of aquifers). Another significant impact is the reduction of buffer capacity of the soil filter, leaving the surface water and groundwater at the mercy of external harmful agents.

In agricultural soils implies a reduction in fertility due to phenomena occurring in organic matter breakdown and nutrient loss. In agricultural soils, acidification leads to a loss of vitality of the plants producing the loss and degradation of leaves and ultimately death of the species vegetables. Finally, there is a loss of plant diversity accompanied changes in soil organisms, by encouraging the proliferation of acidophilic species.

1.5- Seasonal variation

Seasonal changes influence trees, ground flora, soil chemistry and biological processes in forest ecosystems. Seasonality shows its affect already on weeks to months scale. Main seasonal processes are growth and population dynamics (Puhe and Ulrich 2001). Soil biological activity and dissolved organic matter (DOM) change during the year; also enzymatic activity increases through the growing season (spring and summer) (Kaiser et al. 2002; Boerner et al. 2005; Niemi et al. 2007). Seasonal changes in the concentration of dissolved organic carbon (DOC) and DOM are influenced by litterfall inputs and soil organic matter content (e.g. Kalbitz et al 2000).). Production of organic acids in soils together with anion atmospheric deposition control seasonal variations of soil solution acidity.

The decomposition process of organic matter results an organic acids (COOH) that moving into the soil releasing H^+ ($COOH \rightarrow COO^- + H^+$) which replaces the base cations (Ca^{2+} , K^+ and Mg^{2+}) in clays surface, getting a more acid soil.

1.6- Aluminium Speciation methods

Al speciation plays a crucial role in Al toxicity. Because of the complex environmental chemistry of Al and a variety of Al species present in environmental samples, speciation of Al is a very difficult task. Numerous analytical techniques have been developed for Al speciation over recent decades, but many of them have limited selectivity. In addition to experimental analytical techniques, computer modelling is a widely used speciation approach in well defined matrices. Due to the very complex nature of environmental solutions computer modelling represents a complementary tool to experimental analytical techniques for speciation of Al. NMR, fluorescence and IR spectroscopy exhibit a high potential for the investigations into the equilibrium of Al complexes and characterization of different Al species in environmental samples (Scancar and Milacic, 2006).

1.6.1- HPLC/IC

An emerging trend is towards the development of more selective analytical techniques for speciation of Al in environmental samples by applying chromatographic techniques. Ion chromatography, cation-exchange HPLC, cation- and anion-exchange FPLC techniques and size exclusion are combined on-line or off-line with different detection techniques (atomic spectrometric methods, mass spectrometry, specific reactions with chelating agents) for the determination of separated Al species. More selective determination of individual Al species was obtained. The improved selectivity and reliability of the determination (Scancar and Milacic, 2006).

1.6.1.1- Mobile phase

The mobile phase in HPLC refers to the solvent being continuously applied to the column, or stationary phase. The mobile phase acts as a carrier for the sample solution. A sample solution is injected into the mobile phase of an assay through the injector port. As a sample solution flows through a column with the mobile phase, the components of that solution migrate according to the non-covalent interactions of the

compound with the column. The chemical interactions of the mobile phase and sample, with the column, determine the degree of migration and separation of components contained in the sample (Snyder, 1983).

1.6.1.2- Stationary phase

The stationary phase in HPLC refers to the solid support contained within the column over which the mobile phase continuously flows. The sample solution is injected into the mobile phase of the assay through the injector port. As the sample solution flows with the mobile phase through the stationary phase, the components of that solution will migrate according to the non-covalent interactions of the compounds with the stationary phase. The chemical interactions of the stationary phase and the sample with the mobile phase, determines the degree of migration and separation of the components contained in the sample.

1.6.1.3- Injectors

Samples are injected into the HPLC via an injection port. The injection port of an HPLC commonly consists of an injection valve and the sample loop. The sample is typically dissolved in the mobile phase before injection into the sample loop. The sample is then drawn into a syringe and injected into the loop via the injection valve. A rotation of the valve rotor closes the valve and opens the loop in order to inject the sample into the stream of the mobile phase. Loop volumes can range between 10 μL to over 500 μL . In modern HPLC systems, the sample injection is typically automated (Willard, 1988).

1.6.1.4- Pumps

There are several types of pumps available for use with HPLC analysis, they are: Reciprocating Piston Pumps, Syringe Type Pumps, and Constant Pressure Pumps. Reciprocating Piston Pumps consist of a small motor driven piston which moves rapidly back and forth in a hydraulic chamber that may vary from 35-400 μL in volume. On the

back stroke, the separation column valve is closed, and the piston pulls in solvent from the mobile phase reservoir. On the forward stroke, the pump pushes solvent out to the column from the reservoir. A wide range of flow rates can be attained by altering the piston stroke volume during each cycle, or by altering the stroke frequency. Dual and triple head pumps consist of identical piston-chamber units which operate at 180 or 120 degrees out of phase. This type of pump system is significantly smoother because one pump is filling while the other is in the delivery cycle.

Syringe Type Pumps are most suitable for small bore columns because this pump delivers only a finite volume of mobile phase before it has to be refilled. These pumps have a volume between 250 to 500 mL. The pump operates by a motorized lead screw that delivers mobile phase to the column at a constant rate. The rate of solvent delivery is controlled by changing the voltage on the motor.

In Constant Pressure Pumps the mobile phase is driven through the column with the use of pressure from a gas cylinder. A low-pressure gas source is needed to generate high liquid pressures. The valving arrangement allows the rapid refill of the solvent chamber whose capacity is about 70 mL. This provides continuous mobile phase flow rates.

1.6.1.5- Detectors and detector limits

The detector for an HPLC is the component that emits a response due to the eluting sample compound and subsequently signals a peak on the chromatogram. It is positioned immediately posterior to the stationary phase in order to detect the compounds as they elute from the column. The bandwidth and height of the peaks may usually be adjusted using the coarse and fine tuning controls, and the detection and sensitivity parameters may also be controlled (in most cases). There are many types of detectors that can be used with HPLC. Some of the more common detectors include: Refractive Index (RI), Ultra-Violet (UV), Fluorescent, Radiochemical, Electrochemical, Near-Infrared (Near-IR), Mass Spectroscopy (MS), Nuclear Magnetic Resonance (NMR), and Light Scattering (LS).

2- MATERIALS AND METHODS

The aim was to determine the existing differences in soil components between spring and autumn seasons, in others words, the goal was studied the seasonal variability. A set of 16 soil samples from the forest was used of, which 8 were got in autumn and the others 8 were gathered in spring. Each one of the 8 samples from different areas.

The sampling was:

Sample	Locality	Classification	Elevation
1	Smedava (Jizera Mountains)	entic podzol PZ Bedrock-granidiorite	840 a s.l
2	Polesí (natural meadow in Luzické Mountains)	Cambisol CM Bedrock- sandstone and marlite	370 a s.l
3	Horní Sedlo (naturak meadow in Jizera Mountains)	Cambisol CM Bedrock-granidiorite	390 a s.l
4	Bílý Potok (naturak meadow in Jizera Mountains)	entic podzol PZ Bedrock-fluvial sandstonegravel	380 a s.l
5	Dolní Oldrís (hill Tisovec is an agriculturally use meadow in Jizera Mountains)	Cambisol CM Bedrock-granidiorite	420 a s.l
6	Albrechtice (is an agriculturally use meadow in Jizera Mountains)	Cambisol CM Bedrock-biottic granite	510 a s-l
7	Lysý (is an agriculturally use meadow in Jizera Mountains)	Cambisol CM Bedrock-biottic granite	600 a s.l
8	Horní Rasnice (is an agriculturally use meadow in Jizera Mountains)	Cambisol CM Bedrock-profiric granis- granodiorite	390 a s.l

Table 1. *Sampling (locality, classification and elevation)*

The study was divided into two parts. The first part consists of extraction methods, while second part was based on Al speciation and inorganic anions in soil extract using HPLC/IC. All the samples were dried and passed through 2-mm sieves.

2.1- Soil pH in water suspension

Principle of the method: consists of soil pH water suspension describes actual state of the activity of H⁺ (or more precisely H₃O⁺) cations in soil. It is measured potentiometrically.

$$pH = -\log[H_3O^+]$$

Equipment: 50 mL beaker, glass stick, pH-meter with electrode.

Reagent: distilled water boiled for five minutes to remove CO₂ and cooled.

Procedure: consists on weighting 10 g of dry soil into a 50 mL beak and stir for 5 minutes with a glass stick. Submerge the electrode (be careful with the glass electrode), wait a little for an equilibrium and record the pH.

Assessment:

pH H ₂ O	Description
< 4.9	Strongly acid
4.9-5.9	Acid
5.9-6.9	Weakly acid
6.9-7.1	Neutral
7.1-8.0	Weakly alkaline
8.0-9.4	Alkaline
> 9.4	Strongly alkaline

Table 2. Assessment soil pH in water

2.2- Soil pH in KCl 1M suspension

Principle of the method: consists of soil pH water suspension describes actual state of the activity of H⁺ (or more precisely H₃O⁺) cations in soil. It is measured potentiometrically.

$$pH = -\log[H_3O^+]$$

Equipment: 50 mL beaker, glass stick, pH-meter with electrode.

Reagent: KCl 1 M solution.

Procedure: consists on weighting 10 g of dry soil into a 25 mL, shaking during 45 minutes, resting 15 minutes and filtration. Submerge the electrode (be careful with the glass electrode), wait a little for an equilibrium and record the pH.

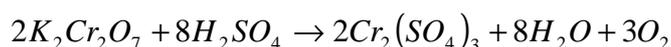
Assessment:

pH KCl	Description
< 4.5	Strongly acid
4.5-5.5	Acid
5.6-6.5	Weakly acid
6.6-7.2	Neutral
> 7.2	Alkaline

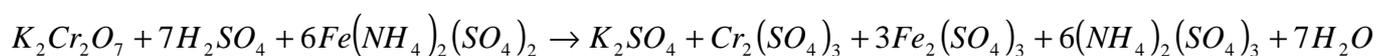
Table 3. Assessment soil pH in KCl.

2.3- Organic carbon content by modified Tyurin's method

Principle of the method: Organic matter is oxidized by potassium dichromate in sulphuric acid:



Remaining dichromate is determined by potentiometric titration:



Reagents:

Reagent A: dichromate solution (0.0667 M $K_2Cr_2O_7$): dissolve 19.6148g $K_2Cr_2O_7$ in 400 mL of distilled water, cooling all the time gradually add 500 ml of concentrated H_2SO_4 and add distilled water up to 1000 mL.

Reagent B: (0.1 M $Fe(NH_4)_2(SO_4)_2$): Dissolve 39.21g $Fe(NH_4)_2(SO_4)_2 \cdot H_2O$ in approximately 300 mL distilled water acidified with 20 mL concentrated H_2SO_4 and add distilled water up to 1000 mL.

Procedure:

1. Grind a small amount of soil and sieve it through 0.25 mm sieve. Weight 0.2 to 0.4 (according to expected Corg content (less if soil is rich in organic matter) of this soil to a beaker (100 mL). Record exact weigh (N, mg).
2. Add 10 mL of dichromate solution (reagent A), cover and shake softly. Put into oven heated to approximately 140°C. Decrease the temperature to 125°C and let react for 45 minutes.
3. Take out, wash the covering glass and walls of the beaker with distilled water
4. Titrate potentiometrically with reagent B on a stirrer to “dead stop”. Record the consumption (S, mL).

Notes:

1. The weight of sample to be used is determined usually according to the colour of soil: darker colour means in most cases higher organic matter content.
2. Two or three blank samples (reagent A with no soil) should go through the same procedure. Reagent B consumption (a, mL) is then used for the factor f.
3. Avoid a long opening of the oven.
4. Adding of distilled water before the titration does not affect the result. The electrodes must be submersed and should not disable stirring.
5. Titration reaction is accompanied by a colour change from orange to blue-green.

Calculations:

Factor of reagent B: $f = 40/a$

Organic carbon content: $C_{org} = (12 - 0.3 * S * f) * 100 / N [\%]$

Where:

f...factor.

a...mean consumption for blank sample titration (mL).

S...consumption for titration of the sample (mL).

N...sampleweight (mg).

2.4- Spectrophotometric method for humus quality

Principle of the method: extractable humic compounds are extracted by alkaline reagent. Absorbance (A) of resulting solution in visible spectral range is measured. Humic compounds of higher quality (more polymerised or condensed) have higher absorbance at longer wavelengths. (Lambert's-Beer's law: for $A < 1$, $A = \epsilon \cdot c \cdot l$, where ϵ is absorption coefficient, c concentration and l the thickness of the layer).

Equipment: Erlenmeyer's flasks 100mL (or PE bottles), shaker, filtration apparatus, paper filters, spectrophotometer with cell.

Reagents: Sodium pyrophosphate (0.05M $\text{Na}_4\text{P}_2\text{O}_7$, $\text{pH} \approx 12$). Dissolve 22.3 g $\text{Na}_4\text{P}_2\text{O}_7 \cdot 10 \text{H}_2\text{O}$ in approximately 800 mL of distilled water. If necessary adjust pH to 12 with 1M NaOH and add distilled water up to 100mL.

Extraction procedure: shake 2g of soil (<0.25 mm) with 40 mL 0.05M $\text{Na}_4\text{P}_2\text{O}_7$ in 100 mL Erlenmeyer's flasks (or PE bottles) for 1 hour. Centrifuge and keep the supernatant measurement.

Spectrophotometric measurement: get baseline using clear 0.05M $\text{Na}_4\text{P}_2\text{O}_7$. Then measure the extract solution. If necessary, dilute with 0.05M $\text{Na}_4\text{P}_2\text{O}_7$, so that absorbance at 400 nm was lower than 1 (or at least not higher than 1.3). record the absorbance at 400 and 600 nm (A_{400} and A_{600} , respectively).

Calculations:

Humus quality parameter: $Q_{4/6} = A_{400} / A_{600}$

The higher the value, the worse is the humus quality.

2.5- Exchangeable and water soluble forms of Al

For the extraction of exchangeable and water soluble forms of Al. To get it, KCl solution and pure water was used. KCl concentration of $0.5 \text{ mol} \cdot \text{l}^{-1}$ and the ratio of solid to liquid was in both cases 1:10 (5g : 50 ml). After that the samples were shaken during 24 hours. Extracts were separated for further analytical treatment from suspension by centrifugation (15 min; 13500 rpm) and finally further purified by filtration through disc filters (filtrak 390 Φ 12,5 cm) and 0,45 μm nylon membrane filter (Cronus Membrane Filter Nylon, GB).

HPLC/IC was used for the detailed Al speciation. The methods separates Al exchangeable forms according to the value of their positive charge.

2.6- Inorganic anions extraction

The samples were extracted by deionised water (ratio soil/water 1:10 w/v, 24 hours extraction on a reciprocal shaker at stable laboratory temperature). The suspensions were then centrifuged at 13500 rpm for 15 min; finally, extracts were filtrated through 0.45 μm nylon membrane filter (Cronus Membrane Filter Nylon, GB). In these aqueous extracts we have determined:

Inorganic anions extracted were ($(\text{CH}_3\text{COO})^-$, $(\text{HCOO})^-$, $(\text{COO})_2^{2-}$, F^- , Cl^- , Br^- , NO_3^- , NO_2^- , $(\text{PO})_4^{3-}$, $(\text{SO})_4^{2-}$). These anions were determined by means of ion-exchange chromatography with suppressed conductivity. . The ion chromatograph ICS 90 (Dionex, USA) equipped with IonPac AS14A (Dionex, USA) guard and analytical columns was used. The eluent composition was 8.0 mM Na_2CO_3 /1.0 mM NaHCO_3 and flow rate was set to 1 mL/min. To suppress eluent conductivity the AMMS 300 – 4 mm suppressor (Dionex, USA) and 25 mM H_2SO_4 reagent was used. The eluent conductivity was even further suppressed by carbon removal device CRD 300 - 4 mm (Dionex, USA) and 0.2 M NaOH solution.

Total amount of Al (Al_{H_2O}). This was done by means of inductively coupled plasma-optical emission spectrometer (ICP-OES, An iCAP 6500 Radial ICP Emission spectrometer; Thermo Scientific, GB) under standard conditions.

The Al speciation was performed. This speciation was carried out under controlled stable temperature 25° C by means of high performance liquid chromatography equipped with ionic column (HPLC/IC) technique.

2.7- Others chemical components and parameters

Previously basic characteristics were determined by commonly use methods:

N_{tot} , S_{tot} , C_{tot} , C_{biom} , nmv, V...

Data obtained from previous analysis were used in the statistical analysis to study its influence on the amount of aluminium in soil.

3- RESULTS AND DISCUSSION

3.1- Soil description according to the Basic soil characteristics and the main anions

The studies were located in different areas of the Jizera Mountains strongly affected by acid deposition which was the cause of forest decline. Soils were identified in most cases as Podzols (Haplic or Entic) and Cambisols (mainly Dystric). All soils were formed on granite bedrock, so that the effect of geology on the spatial variation of soil characteristics can be neglected.

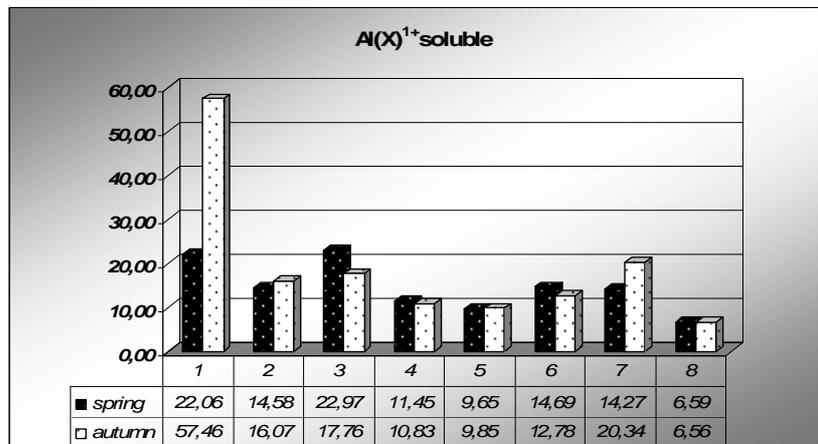
Meadow areas formed after forest decline due to acid deposition, often populated by grass (mainly *Calamagrostis villosa*). Soils under this grass cover have often better chemical properties than soils covered by forests due to better quality of grass litter, its influence on N cycling, organic matter turnover and base cations leaching. Liming, used most often as a chemical amelioration measure, usually results in pH increase, higher base saturation, and a decrease of the solubility of Al and most other risk elements.

The analysis showed sulphur level above normal in comparison with other basic elements of soil (for example: N_{tot}, C_{tot} which retain relatively low values). High content of S is caused by large input of these elements from atmospheric deposition and by their incorporation into organic matter.

Low pH values were found in both water [4.27;5.61] and KCl [3.66;5.32] extractions classifying the soil between acid a strongly acid. The humus quality ($Q_{4/6}$) was also analyzed recording a low quality with values distant to 1, resulting a poor soil structure and consequently faster depletion of base cations in soil.

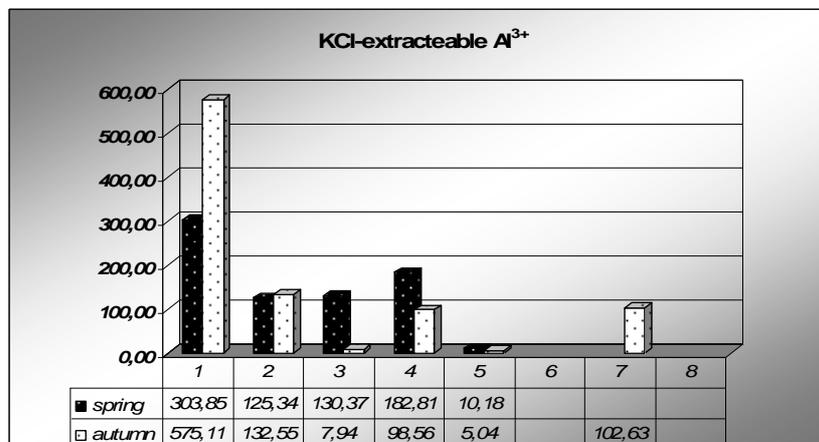
3.2- Differences between Al contents and speciation in different localities

Al forms were separated into three different groups according to their charge: $[Al(X)^{1+}, Al(Y)^{2+}$ and $Al^{3+}]$ and studied in the different sampling points in Al_{H_2O} (soluble forms) and Al_{KCL} (exchangeable form) extractions. Both in the aqueous and KCl-exchangeable extract, the lower part of Al is in the $Al(X)^{1+}$ fraction, representing mainly Al bound in organic complexes, which is a negative result indicating rather high actual Al toxicity.



Graphic 1. Amount $Al(X)^{1+}$ soluble in each area (mg/kg soil dry).

Most of KCl-extractable Al is in the most dangerous fraction of Al^{3+} was observed in the first four areas although the highest dates (until approximately 575 mg Al^{3+} /kg soil dry were found in the area 1 (located 831 a.s.l.).



Graphic 2. Amount Al^{3+} exchangeable in the first four areas (mg/kg soil dry).

This could be explained by evaluating the relationship between temperature and the amount of aluminium in soil, specifically the aluminium solubility in function of temperature variation.

(Mladkova et al., 2004) showed, using traditional statistical approach, that the content of KCl-extractable (exchangeable) Al forms is influenced mainly by altitude, while the content of Na₄P₂O₇-extractable (mainly organically bound) Al forms is influenced more by forest type.

It might be influenced by higher altitudes which are associated with more severe conditions like lower temperatures leading to stronger weathering and increased Al solubilisation (Drabek et al., 2001). Therefore be logical to find larger amounts of Al in colder areas such as the Area1 (located to 831 meters), the remaining areas are located at lower altitudes.

3.3- Al (different form and species) relationship with soil characteristics

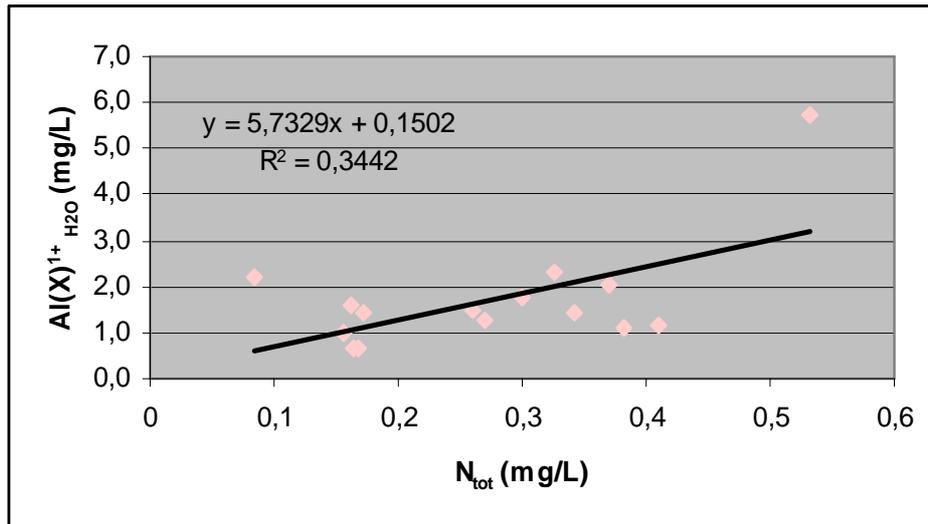
This section aims to study the relationships among basic soil characteristics and different species of aluminium. This analysis is done through a simple regression among the variables, observing the correlation coefficients among these and the significance level obtained from the statistical analysis of regression.

Only significant relationships will be shown, in other words, those in which we obtained a p-value <0.05 statistical analysis. The following table shows the p-values amount the variables compared:

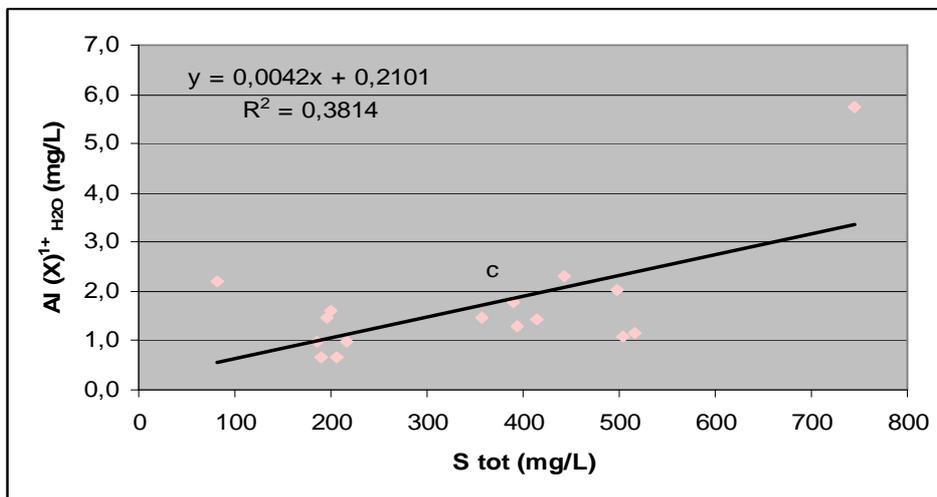
p-value	N _{tot}	S _{tot}	C _{tot}	C _{biom}	C _{ox}	Q _{4/6}	pH _{H2O}	pH _{KCL}
Al(X) ¹⁺ _{H2O}	0.0169	0.0108	0.0001	0.3393	0.0001	0.8985	0.0013	----
Al(Y) ²⁺ _{H2O}	0.8047	0.8378	0.8737	0.8247	0.9231	0.3484	0.2364	----
Al ³⁺ _{H2O}	0.8624	0.9079	0.8513	0.6086	0.8261	0,7831	0,2368	----
Al(X) ¹⁺ _{KCl}	0,0105	0.0073	0.0001	0.3255	0,0001	0.6141	----	0,0152
Al(Y) ²⁺ _{KCl}	0,2101	0.2347	0.7425	0.0283	0.7483	0.4724	----	0.9682
Al ³⁺ _{KCl}	0.1494	0.1373	0.0093	0.9160	0.0115	0.7615	----	0.0066

Table 4. P-value among the variables analyzed

The following equations allow us to observe the relationship between variables so as to establish the degree of correlation by a linear fit. Graphics 3 and 4 show significant correlations between $Al(X)^{1+}$ soluble and N ($R^2= 0,3442$) and S ($R^2=0,3814$). High content of S and N is caused by large input of these elements from atmospheric deposition and by their incorporation into organic matter.



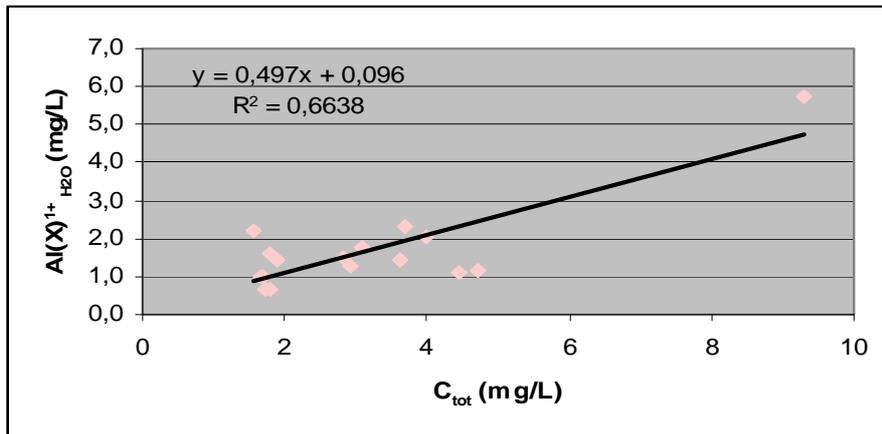
Graphic 3. Relation between amount $Al(X)^{1+}$ soluble and N tot in soil.



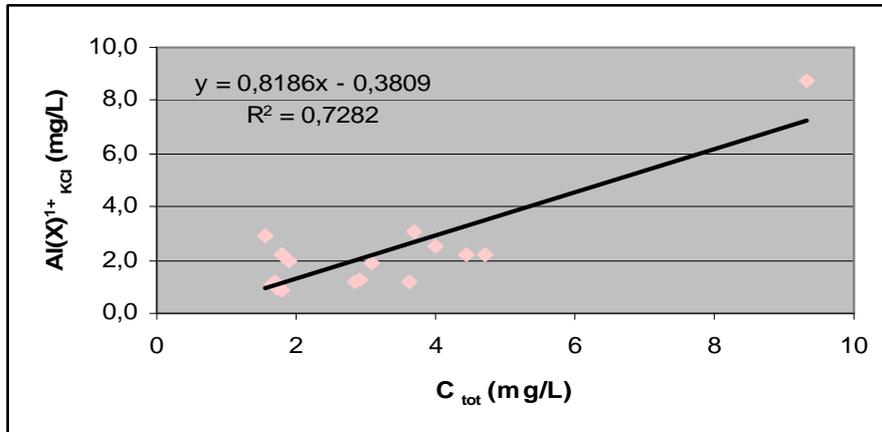
Graphic 4. Relation between amount $Al(X)^{1+}$ soluble and S tot in soil.

The same goes for $Al(X)^{1+}$ exchangeable where get correlation coefficients around $R^2= 0.4121$ for N and $R^2 =0.3838$ for S.

On the other hand, higher correlation coefficient is observed both Al(X)^{1+} soluble ($R^2 = 0,6638$) and Al(X)^{1+} exchangeable ($R^2 = 0,7282$) in relation to C_{tot} , showing the relationship of this aluminium species with organic matter. The following graphics (5 and 6), can be seen this relationship as well as the adjustment equations in each case:



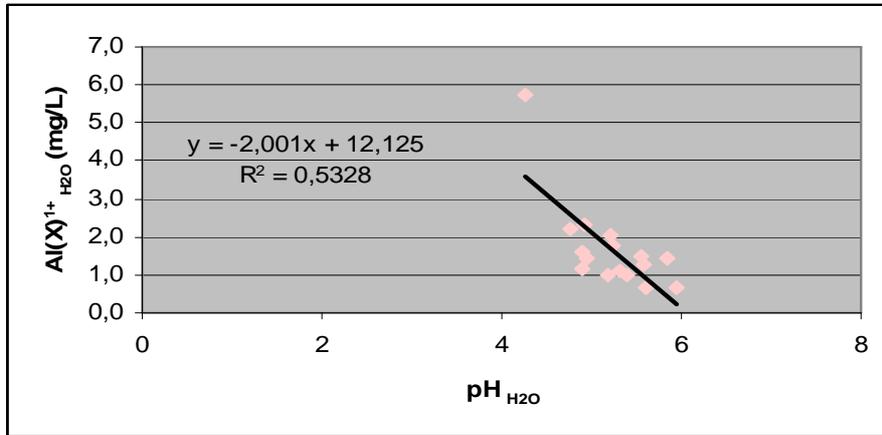
Graphic 5. Relation between amount Al(X)^{1+} soluble and C_{tot} in soil.



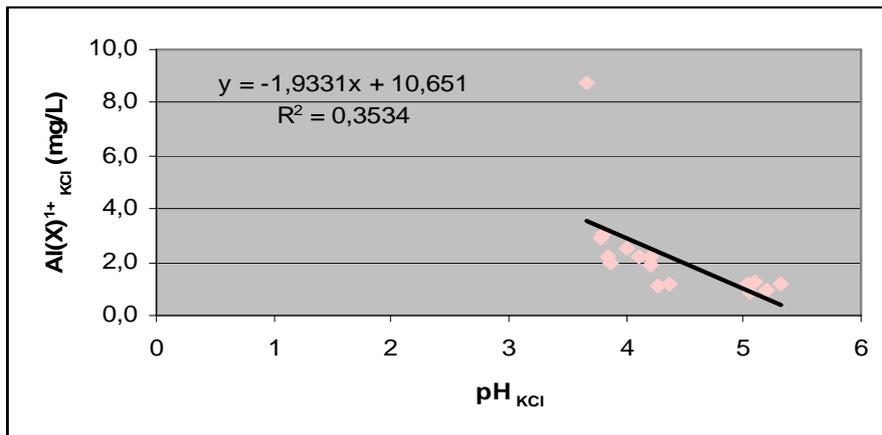
Graphic 6. Relation between amount Al(X)^{1+} exchangeable and C_{tot} in soil.

Analyzing the results generally, positive relations are found for carbon, nitrogen and sulfur, it means, aluminium quantities increase proportionately according to this basic soil elements concentration.

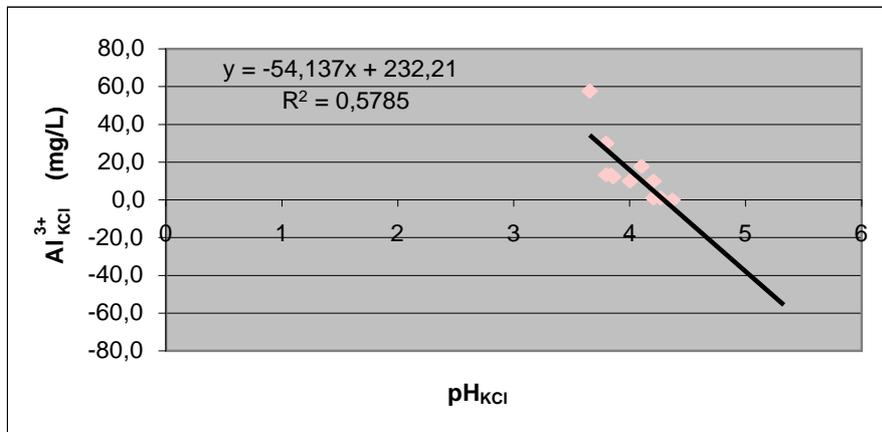
However, the relation between pH and Al species concentration is negative, in others words, the highest aluminium values are found at low pH. It can be observed in the following Graphics:



Graphic 7. Relation between amount $Al(X)^{1+}$ soluble and pH in soil.



Graphic 8. Relation between amount $Al(X)^{1+}$ exchangeable and pH in soil.



Graphic 9. Relation between amount Al^{3+} exchangeable and pH in soil

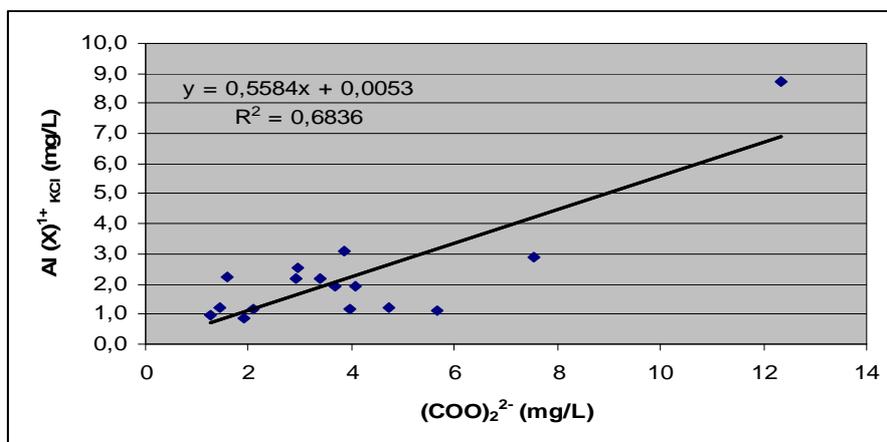
3.4- Al (different form and species) relationship with the content of main anions

The same way, the significant relationships in the anions among the different aluminium species will be studied with the intention of establishing linear relationships among them. The following table shows the p-values amount the variables compared:

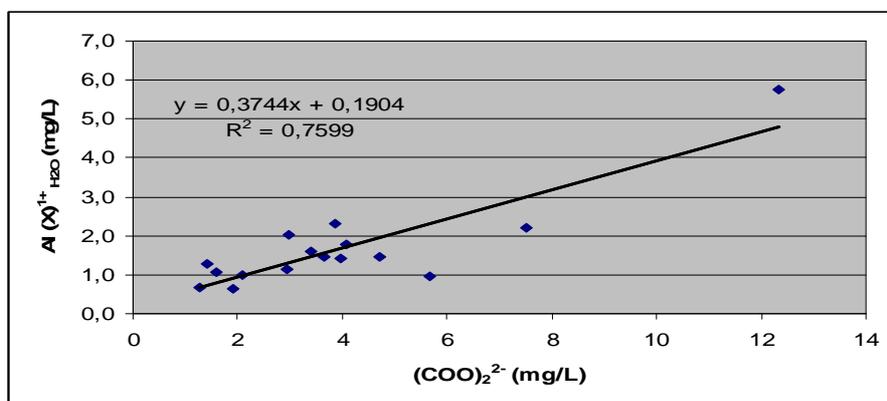
p-value	CH ₃ COO ⁻	HCOO ⁻	(COO) ₂ ²⁻	NO ₂ ⁻	NO ₃ ⁻	SO ₄ ²⁻	Cl ⁻	F ⁻
Al(X) ¹⁺ _{H₂O}	0.5582	0.1196	0,0001	0.7015	0,4966	0.4810	0.3028	0.8815
Al(Y) ²⁺ _{H₂O}	0.4631	0.1269	0.9018	0.8076	0.5593	0.8472	0.2746	0.8841
Al ³⁺ _{H₂O}	0.7205	0.2111	0,5257	0.6676	0.7043	0.9337	0.2322	0.8036
Al(X) ¹⁺ _{KCl}	0.4434	0.1118	0.0010	0.9204	0.5016	0.5568	0.3088	0.7155
Al(Y) ²⁺ _{KC}	0.1846	0.1886	0.1010	0.1882	0.1309	0.9664	0.7138	0.3633
Al ³⁺ _{KCl}	0.4941	0.1118	0.0010	0.1882	0.2811	0.6799	0.4316	0.3633

Table. P-value among the variables analyzed

The Al(X)¹⁺ soluble and exchangeable shows a direct relation with organic matter decomposition both with p-value < 0.05. This decomposition process forms organic acids increasing de soil acidification. Therefore is logical to find this relation because Al(X)¹⁺ specie is usually related with organic complexes (graphics 10 and 11).



Graphic 10. Relation between amount Al(X)¹⁺ soluble and (COO)₂²⁻ in soil.



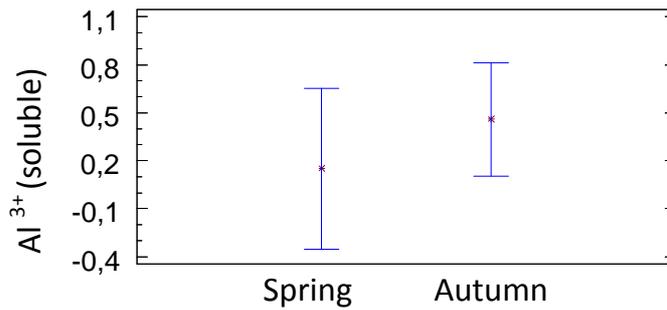
Graphic 11. Relation between amount Al(X)¹⁺ exchangeable and (COO)₂²⁻ in soil.

3.5- Differences between spring and autumn in regard to the Al contents and speciation

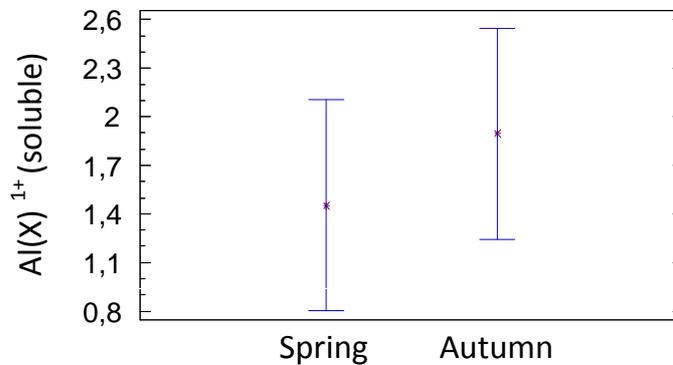
The seasonal study does not show significant differences regarding the quantities of aluminium analysed, in spite of the fact that initially, one might expect higher aluminium levels in Spring, due to the influence that temperature has on this element. This could be caused by the elimination by Spring rains of the layer of superficial snow, which prevents its filtration into the ground, and its passing to lower levels.

The following graphs show the results obtained:

3.5.1 Al (H₂O- soluble)

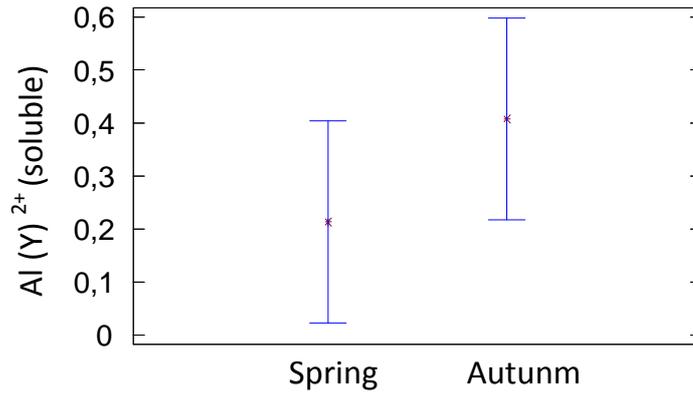


Graphic. Al³⁺-soluble differences between spring and autumn (mg/L).



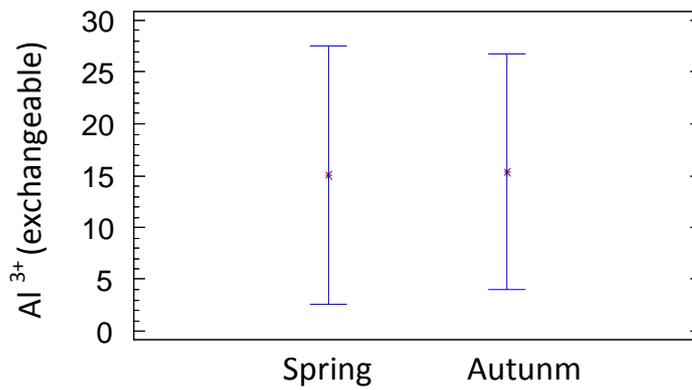
Graphic. Al(X)¹⁺-soluble differences between spring and autumn (mg/L)..

Soil acidification with a specific view to aluminium

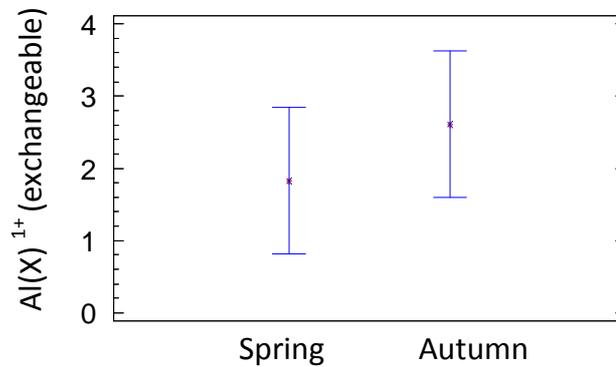


Graphic. $Al(Y)^{2+}$ -soluble differences between spring and autumn (mg/L).

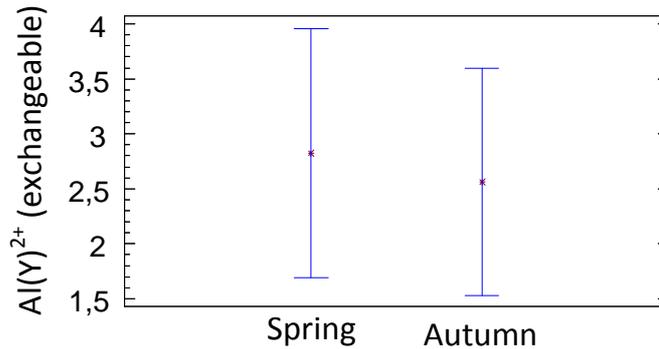
3.5.2- Al (KCl-exchangeable)



Graphic. Al^{3+} -exchangeable differences between spring and autumn (mg/L).



Graphic. $Al(X)^{1+}$ -exchangeable differences between spring and autumn (mg/L).



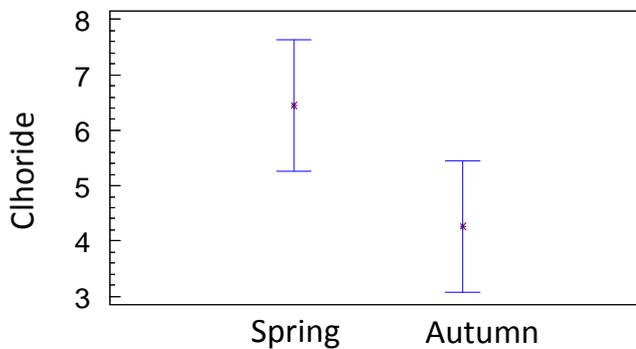
Graphic. $Al(Y)^{2+}$ -exchangeable differences between spring and autumn (mg/L).

3.6 Differences between spring and autumn for basic characteristics and main anions content

As in the previous point, no significant difference was found when comparing seasonally ions. However, seasonal difference is almost significant for chloride ion, the results are slightly higher in spring.

An explanation that could justify the levels of chloride found could be the use of salt which occurs in these areas during the winter. This substance is used to avoid the accumulation of snow in certain places and the consequent problems this causes. Due to this, it is logical that high chloride levels can be found in Spring, as all this accumulated salt becomes a part of the ground.

It can be seen in the following graphic:



Graphic. Cl^- differences between spring and autumn (mg/L).

4.- CONCLUSIONS

-No difference was found in the seasonal analysis among different species of aluminium probably because elimination by Spring rains of the layer of superficial snow, which prevents its filtration into the ground.

- Slightly higher chloride levels were found in spring because this substance is used to avoid the accumulation of snow in certain places and the consequent problems this causes although the difference is not significant.

- Highest levels of aluminium observed belong to Al^{3+} which is the most dangerous aluminium form.

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