Diamagnetic Susceptibility and Root Growth Responses to Magnetic Fields in *Lens culinaris*, *Glycine soja*, and *Triticum aestivum*

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ABSTRACT

We studied the response of root growth to different magnetic fields and forces. We submitted the seeds of three plant species, *Lens culinaris* L., *Glycine soja* Siebold & Zucc., and *Triticum aestivum* L., which differ in concentrations of paramagnetic (e.g., Fe or Co) and diamagnetic materials (e.g., starchy amyloplasts), to different static magnetic fields and forces. A magnetic field of 176 G reduced root growth of *L. culinaris*, *G. soja*, and *T. aestivum*, 37, 31, and 15%, respectively. A weaker magnetic field of 21 G reduced root growth of *L. culinaris* and *G. soja* only 13 and 21%, respectively, whereas it had no significant effect on the cereal *T. aestivum*. The germinating seeds of *L. culinaris* and *G. soja* were less diamagnetic than *T. aestivum*, and the latter had a smaller paramagnetic component. Since at room temperature, the paramagnetic

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component was much smaller than the diamagnetic one, the magnetic inhibition of root growth may be linked to the diamagnetic susceptibility, the inhibition being greater for the less diamagnetic materials and for the stronger magnetic forces. These results provide new examples of possible species-specific effects of moderate magnetic fields on plant growth, especially when growth is rapid, such as root growth after germination. We propose a simple hypothesis to relate root growth inhibition with magnetic fields and with the different responses found among species, seasons, and physiological and environmental conditions reported here and in the literature. It is based on a reduced magnetic force acting on the cell biological substances and on the cellular organelles such as amyloplasts, rather than on the cytoplasmic matrix where they are immersed as a consequence of their lower diamagnetic susceptibility. As a result, a nonuniform magnetic field exerts a ponderomotive force on the biological components in the opposite direction to the growing tip. This can result in intracellular magnetophoresis, and can account for inhibition of the root growth rate downwards. This inhibition would be stronger the lower the diamagnetic susceptibility.

Key Words: Static magnetic fields; Root growth; Diamagnetic susceptibility; Germinating seeds; *Lens culinaris; Glycine soja; Triticum aestivum*.

INTRODUCTION

Over many years, the effects of magnetic fields on plant life have been the subject of several studies. As early as 1930, Savostin reported a 100% increase in the rate of elongation of wheat seedlings under the influence of a magnetic field. Later, Murphy (1942) reported changes in seed germination, and Audus (1960) and Pittman (1965) reported a strong magnetotropic effect on root development. Audus (1960) found that actively growing roots could be induced to curve away from regions of high to regions of low magnetic flux (i.e., down a steep magnetic gradient under conditions in which geotropic response had been eliminated on a clinostat).

More recently, many authors have reported the effects of static magnetic fields on the metabolism and growth of different plants (Dulbinskaya, 1973; Kato, 1988; Kato et al., 1989; Peteiro-Cartelle and Cabezas Cerato, 1989; Pittman, 1977). Some decades ago, strong magnetic fields were used (Dunlop and Schmidt, 1969; Pittman, 1977) but more recently, moderate fields, even as small as the geomagnetic field, have been reported to produce striking effects (Kato, 1988; Kato et al., 1989; Peteiro-Cartelle and Cabezas-Cerato, 1989). Smith et al. (1993) found that using different field combinations, one could separately alter the root mass, leaf size, and stems thickness. Roots seem much more susceptible to the magnetic field than shoots (Kato, 1988; Kato et al., 1989). The reported effects include growth stimulation and inhibition depending on the species and its physiological state (Krizaj and Valencic, 1989). No proper physiological explanation has been provided so far for all these effects (Phirke et al., 1996).

The magnetization $M$ of a substance is proportional to the magnetizing (external) magnetic field $H$, thus $M = \chi H$. The proportionality factor is the magnetic susceptibility ($\chi$). The vast majority of biological substances have negative $\chi$ (are diamagnetic), except for some proteins that contain metal ions, such as
hemoglobin, cytochrome, or ferritin, which can be paramagnetic (Piruzyan et al., 1980; Theil, 1987).

Metals such as Fe and Co are present in plants in low concentrations as trace elements. They are generally considered as *partially mobile* physiologically. The presence of a magnetic field may affect a normal tendency of Fe and Co to collect in the meristems of the roots. Then, as these meristems begin to move out of the field, the forces exerted could also affect in an opposite way the continued translocation of the microelements into the root meristems, thereby producing the altered growth rate pattern exhibited by the roots in magnetic fields (Mericle et al., 1964).

It is also known that biological systems employ special cells called statocytes to “sense” gravity. These statocytes can contain mineral masses (e.g., sand grains in some crustaceans or CaCO₃ in mammals). Plant statocytes typically contain starchy amyloplasts. In roots of higher plants, amyloplasts are mostly localized in the root cap. The diamagnetic susceptibility of starchy amyloplasts is greater than that of cytoplasm (Piruzyan et al., 1980). Therefore nonuniform magnetic fields exert a ponderomotive force on amyloplasts which can result in intracellular magnetophoresis (motion). Thus, plants could perceive a magnetic field of sufficient intensity and gradient and respond to the resulting amyloplast displacement as they do to gravity. Audus (1960), Audus and Whish (1964), and Schwarzacher and Audus (1973) used high gradient magnetic fields to induce curvature in roots, coining the term magnetotropism for it, although it seems that the cause of the growth response is a ponderomotive force and not the magnetic field.

In this article we hypothesize that plants with different concentrations of diamagnetic substances and organelles, such as starchy amyloplasts or paramagnetic elements (such as Fe or Co) would present different magnetic susceptibility and different responses to magnetic forces. To test this hypothesis we measured the magnetic susceptibility of germinating seeds and the root growth response to different magnetic fields and forces in lentils (*Lens culinaris*), soy beans (*Glycine soja*), and wheat (*Triticum aestivum*), which were selected to have different magnetic susceptibilities and thus likely different sensitivities to external magnetic fields. We aimed to develop a simple hypothesis to relate the effect of external magnetic fields on root growth to the different sensitivities of the diverse species.

**MATERIALS AND METHODS**

**Plant Material and Growth Conditions**

Several sets of experiments were conducted throughout a year with *L. culinaris* Medik., *G. soja* Siebold & Zucc., and *T. aestivum* L. seeds. To allow free root growth, seeds of the three species were soaked in water and grown on moist paper in empty cuvettes. The cuvettes (Fig. 1) were kept in a seed germination chamber (INDAF, humidifier Mod. AIR-BLUE H-1 N° 101.324, Barcelona, Spain) at 25°C with a photoperiod of 14 h day/10 h dark.
Treatment with Static Magnetic Fields

The static magnetic fields were obtained by using neodymium–iron–boron circular magnets (2 cm external diameter, 1 cm internal diameter, and 5 mm thickness) magnetized along a diameter and attached to the cuvettes (Fig. 1). The nominal magnetic remanence of the magnets was $B_r = 10.60 \text{kG}$.

In a first series of experiments (configuration A), seeds were exposed to only one pole (North Pole of a single magnet) (Fig. 1A). In a second series, seeds of the three species were exposed to the field created by two identical magnets placed at opposite sides of the cuvettes (and thus separated by the 12-mm gap of the cuvette) (Fig. 1B). Magnets were placed with parallel polarity of their magnetization vector (Configuration B). In this magnet configuration B, the horizontal component of magnetic field induction ($B_x$) at the seed position (center of the cuvette) was higher than that of configuration A, but all field gradient components

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**Figure 1.** Diagrams showing growth chambers, magnet experimental setups (A one magnet setup, B two magnets setup), and corresponding vertical distribution of magnetic induction $B_z$ (G). The bold arrows indicate the direction of force acting on diamagnetic substances. A diamagnetic body will move away from the gap (Kuznetsov and Hasenstein, 1996). C and D. Magnetic force ($f_z = F_z/\chi = B_x \delta B_z/\delta x + B_z \delta B_z/\delta z$; see text) at different distances from the magnet center.
Diamagnetic Susceptibility and Root Growth Responses

...(\n\[r x = \frac{\partial}{\partial x} \text{ and } r z = \frac{\partial}{\partial z}\) were smaller (Fig. 1). We measured the magnetic induction in the cuvette axis, by moving a calibrated Hall probe at successive points along a line passing through the center of the air gap between the poles. In Fig. 1 we show $B_z$ for both magnet configurations. The magnetic inductions at the seed position ($x = 6$ mm) were about 175 G and 21 G for (A) and (B), respectively. Data in Fig. 1 clearly indicate that in configuration B, the vertical component of the unitary magnetic force ($f_z = B_x \frac{\delta B_z}{\delta x} + B_z \frac{\delta B_z}{\delta z}$ up to 2395 G$^2$/mm at 8 mm) was substantially lower than that obtained in configuration A ($f_z$ up to 54,000 G$^2$/mm at 4 mm).

In each one of the experiments (two magnetic configurations [one and two magnets] for each one of the three species), a set of 5 cuvettes with identical magnets were used. We also conducted controls consisting of the same experimental devices and measurements in 5 cuvettes but with dummy, nonmagnetized Neodymium magnets of the same dimensions, geometry and orientation. The object of these controls was to check and eliminate the possibility of gradients, other than magnetic, associated with the experimental setup. The experiments were replicated 4 times over a time interval of 10 months (January, February, April, and October) for all seeds.

Seed Magnetic Susceptibility

To prepare seeds in a form appropriate for magnetic measurements, seeds that were starting to germinate were dried at 70°C. Next, the seeds were finely ground in teflon tubes with a teflon pestle, weighed, and encapsulated in identical plastic bags. Magnetic measurements were performed using a Quantum Design SQUID (Orlando, FL, USA) magnetometer. Susceptibility data reported here were measured under a 5 kOe field from 4 to 300 K. The magnetic contribution of the plastic bag was appropriately subtracted from the raw data.

Elemental Analyses

Seed concentrations of Ca, K, Mg, Al, Fe, Na, Mn, Zn, Pb, Ti, Sr, Ba, Cd, Co, Cu, Mo, Ni, V, Cr, S, P, Si, and B were measured on subsamples (about 100 mg) of dry ground seeds. Samples were digested in a Whirlpool AVM 635 microwave (Barcelona, Spain) using open fluorinated ethylene propylene flasks (Nalge Company, Rochester, UK) with an acid solution of 2:1 nitric and perchloric acids (60%). Once samples were efficiently oxidized (0.5 cm$^3$ remaining), they were diluted with 20 mL of perchloric acid (3%). Flasks were stoppered and shaken by hand for thorough mixing of the contents. The sample solution was poured into a polystyrene sample tub, placed in an autosampler carousel and analysed for the above mentioned 23 elements by Polyscan Thermo Jarrel ASH Model 61 E ICAP-AES (inductively coupled argon plasma emission spectrometer, Offenbach, Germany) (Peñuelas and Matamala, 1993). Quality control was ensured by including a reference sample (Reference materials, Community Bureau of Reference, Commission of the European Communities) every 10 samples analyzed. Readings were accepted when reference samples were within a 5% range of known values for macroelements and 10% range for microelements.
Root Growth

After germination, we measured the root length several times every day while they were growing in each of the growth cuvettes (5 with magnetized magnets and 5 with dummy magnets). The root length 24 h after germination was the dependent variable.

Starchy Amyloplasts

A hand-microtome (Cylinder type, Shibuya Optical Co., Ltd., Tokyo, Japan) was used to obtain transverse sections (10 μm) of root at three different zones, from proximal to distal of the root growing tip. The sections were thereafter colored with iodine solution and observed under the microscope for assessing the number of amyloplasts.

Statistics

Statistical significance was tested with repeated-measures analyses of variance (ANOVA) with the presence or absence of a magnetic field as the main independent variable. One-way ANOVAs were also conducted for each one of the measurement times. One-way ANOVAs and posthoc tests were also conducted for the seed elemental concentrations as dependent variables and the species as independent variable. Statistical analyses (the mentioned ANOVAs and regressions between root growth inhibition and magnetic forces) were conducted using STATISTICA version 5.0 for Windows (StatSoft, Inc., Tulsa, OK). Data were log-transformed to meet normality requirements.

RESULTS

Root Growth Inhibition and Magnetic Force

An inhibitory effect of the magnetic fields on root growth was found in experiments conducted with *L. culinaris*, *G. soja*, and *T. aestivum* in the experimental setup with seeds facing the north pole of only one magnet, under a magnetic field of 176 G (Fig. 1A). The mean decrease in root growth of *L. culinaris* after 1 day was 37% (Fig. 2) (40% in January, 33% in February, 30% in April, and 38% in October) (see detailed results in Fig. 3 as an example of the experiments conducted for all these three species). The decreases in root growth of *G. soja* and *T. aestivum* after 1 day were 31 and 15%, respectively (Fig. 2).

When two magnets were used (Fig. 1B) the mean decrease after 1 day in root growth of *L. culinaris* was smaller, 13% (Fig. 2): 15%, 0% (not significant), 21 and 15%, the 4 times we carried out the experiments (see Fig. 3 as a detailed example). Similar results were found when two magnets were used for *G. soja*. Its root growth was also inhibited an average of 21% (Fig. 2). However, there was no significant root growth decrease for *T. aestivum’s* central root (Fig. 2). This species had three growing roots. The response of the lateral root close to the south pole and the lateral root close to the north pole were identical (data not shown).
The inhibition of the root growth calculated as 
\[
\frac{\text{root length at control}}{\text{root length under magnetic field}} \times \frac{\text{root length at control}}{\text{root length under magnetic field}}
\]
was already maximum at 4–5 mm from the magnets, under maximum magnetic forces.

**Figure 2.** Relationship of root growth inhibition at identical stage of development (1 day) with the diamagnetic component of the seeds of the three studied species for the magnetic induction \(B_z\) 176 G tested in configuration A (one magnet) of Fig. 1.

The inhibition of the root growth calculated as \([\text{root length at control} - \text{root length under magnetic field}] / \text{root length at control}\) was already maximum at 4–5 mm from the magnets, under maximum magnetic forces.

**Magnetic Susceptibility**

Figure 4 shows that all studied seeds do have a temperature-dependent paramagnetic susceptibility \(\chi_p > 0\) superimposed upon a temperature independent diamagnetic \(\chi_d (<0)\) component (Table 1). The presence of a paramagnetic...
Figure 3. (A) Root growth inhibition (24 h after germination) in the three studied species for the two tested magnetic inductions ($B_z$), 21 and 176 G. Bars are standard errors ($n = 4$ data sets of 5 measures each). *$P < 0.01$ significant difference between 21 and 176 G. (B) Diamagnetic component of the germinating seeds. The diamagnetic susceptibility was measured under 5 kOe field from 4 to 300 K using a Quantum Design SQUID magnetometer.
Figure 4. Time-course of root length of *Lens culinaris* in the two tested magnetic inductions $B_z$ 21 and 176 G shown in Fig. 1 and in control not magnetized environment. The experiment was repeated in different months. Bars are standard errors of 5 measurements conducted in 5 different germinating roots.

Table 1. The diamagnetic susceptibility $\chi_d$ (emu g$^{-1}$), the room temperature susceptibility $\chi(300 \text{ K})$ (emu g$^{-1}$), and Curie Constant $C$, (emu$^{-1}$ K g$^{-1}$) of the germinating seeds of the studied species.

<table>
<thead>
<tr>
<th>Species</th>
<th>$\chi_d$</th>
<th>$\chi(300 \text{ K})$</th>
<th>$C$</th>
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<tbody>
<tr>
<td><em>Lens culinaris</em></td>
<td>$-5.6 \times 10^{-7}$</td>
<td>$-5.7 \times 10^{-7}$</td>
<td>$1.1 \times 10^{-6}$</td>
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<tr>
<td><em>Glycine soja</em></td>
<td>$-6.4 \times 10^{-7}$</td>
<td>$-6.2 \times 10^{-6}$</td>
<td>$2.7 \times 10^{-6}$</td>
</tr>
<tr>
<td><em>Triticum aestivum</em></td>
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<td>$4.9 \times 10^{-6}$</td>
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contribution is related to the existence of paramagnetic ions such as Fe, Co, Mn, or Ni, in the roots (Table 2). The diamagnetic response has its origin in the organic matter of the biological substances and organelles, such as amyloplasts. The solid line through the data (Fig. 4) represents the results of the fits using \( \chi(T) = \chi_p(T) + \chi_d \) with \( \chi_p(T) \) given by Curie law \( (= C/T) \). The \( \chi_d \) and \( C \) values extracted from the fits are included in Table 1. This table shows that at room temperature, the diamagnetic component is larger than the paramagnetic, and dominates the overall magnetic susceptibility. We observed that the diamagnetic component was stronger

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and the lowest ones in *T. aestivum*, and although Mn concentrations were lower in the lentils and beans, the total amount of metal was slightly higher in the legumes than in the cereal (Table 2). Table 2 also shows higher concentrations of other elements such as S and Cu in the legumes than in the cereal. These elements provide a smaller paramagnetic contribution to these differences than Fe so it is not expected to be as relevant as that of Fe or Mn given their smaller paramagnetism.

The microscopic observations of the root sections and their starchy amyloplasts did not show any significant differences among species or among different parts of the elongating roots (data not shown). Since optical amyloplasts traits are not the apparent cause of the different diamagnetic properties of those studied species, further, more detailed biochemical analyses such as determination of root amyloplast density and diamagnetic susceptibility, as well as the diamagnetic susceptibility of the other cellular components, are warranted to figure out the drivers of the diamagnetic differences found among these studied species (Table 1).

**DISCUSSION**

For the different magnet configurations (A and B) for which the field inductions were 21 and 176 G at the root position, we have found a clear inhibition of root growth for the legumes *L. culinaris* and *G. soja*, whereas there was no significant effect for *T. aestivum* under the weaker magnetic field of 21 G. There was greater inhibition for the less diamagnetic materials as illustrated in Fig. 5. Furthermore, the observation that the experiments performed with a single magnet, which
produces a stronger (vertical) magnetic field and field gradient, leads to higher inhibition effect (Fig. 2), clearly proves the magnetic origin of the inhibition effect.

To get some insight into the possible microscopic origin of the inhibition effect, we propose here a simple hypothesis relating inhibition to the diamagnetic biological substances and cellular organelles such as amyloplasts \((\chi_b < 0)\) immersed in a diamagnetic cytoplasmic matrix of diamagnetic susceptibility \((\chi_c < 0)\) as illustrated in Fig. 6. In fact, the same approach has been used to explain the magneto-levitation of water and other diamagnetic materials (Beaugnon and Tournier, 1991).

The total vertical force acting on the biological substances and organelles, including the forces associated with both the differences of densities \((\rho_c - \rho_b)\) and \((\chi_b - \chi_c)\), respectively, and the distinct magnetic susceptibilities can be written as in Beaugnon and Tournier (1991):

\[
F = (\rho_c - \rho_b)g + (\chi_b - \chi_c)f_z, \tag{1}
\]

**Figure 6.** Simple model of vertical forces acting on diamagnetic biological substances and cellular organelles immersed in a diamagnetic cytoplasmatic matrix under a magnetic field.
where $f_z$ is the vertical component of the unitary magnetic force $f = (B \times \nabla) \times B$ and $g$ is the acceleration due to gravity. We note that if $|\chi_b| < |\chi_c|$ then the force $|F|$ is smaller (and even changing sign and pointing upwards) than that existing without magnetic field. Therefore, if $|\chi_b| < |\chi_c|$ as it normally happens, since the cytosol has a magnetic susceptibility close to that of water ($-7.2 \times 10^{-7}$ emu g$^{-1}$) (Weast et al., 1989), one can expect some inhibition of the root growth rate downwards, which will be stronger the lower the $\chi_b$.

If we assume that the inhibition rate is proportional to the force $F$, then our data of Fig. 5 displays the behavior predicted by Eq. (1). Therefore our data suggest that this simple hypothesis of magnetic forces acting on the biological substances and cellular organelles immersed in the cell cytoplasm accounts for the observed inhibition effect and thus it can be at its origin.

Our results and hypothesis agree with the suggestion that magnetic fields to cause effects related to interference with cytoplasmatic ion currents or ion distribution (Weisenseel et al., 1992), and with induction of intracellular magnetophoretic displacement of starch-containing plastids (Kuznetsov and Hasenstein, 1996; Kuznetsov et al., 1999) in cells exposed to high-gradient magnetic fields (HGM-F). When subjected to a nonuniform magnetic field, magnetically heterogeneous systems experience ponderomotive forces that depend on their relative magnetic susceptibilities (Kuznetsov and Hasenstein, 1996).

The diversity of responses among the studied species and seasons is in agreement with the existence of several reports on the difficult repeatability of experiments with magnetic fields (Davis and Rawls, 1987; Ruzic et al., 1992). It seems that biological systems during phases of intensive growth, such as those tested here and in suboptimal environmental conditions such as under drought stress (Ruzic et al., 1998a) or under acid environments (Ruzic et al., 1998b), are prone to exhibit greater sensitivity to low fields (Ruzic et al., 1998a,b). The effect seems to depend on the physiological state of the plant tissue (Ruzic et al., 1992). Therefore, the response would depend not only on the magnetic induction and its gradient (Wittekind et al., 1990), but also on the physiological state of the experimental organism, as proposed by Mcleod et al. (1992). This suggests that response to magnetic treatment might occur at different intensities under different environmental conditions.

We need to concentrate on defining the environmental conditions accompanying the response to magnetism. The effects of magnetic fields are superimposed on endogenous rhythms; in some situations leading to inhibition, and in others to stimulation, while sometimes no effects had been reported (Ruzic et al., 1993). However, in our study, the inhibition, although of variable intensity, was consistent for all the different dates (and seasons) in which we conducted the experiment (Fig. 3), perhaps as a consequence of the stable environmental conditions of the tests (and the large fields).

The results presented here add new examples of magnetic effects to the diversity of reported ones such as recent studies showing results of static magnetic fields at the molecular level. Rikken and Raupach (2000) report the first unequivocal use of a static magnetic field to bias a chemical process in favor of production of one of two mirror-image left or right enantiomers. This is an important biological effect, since the chemistry of life is homochiral, being based almost exclusively on $L$-aminoacids and $D$-sugars. The ability of biological molecules to discriminate between
enantiomers is vital for living systems. Our results showing root growth inhibition by magnetic forces and therefore a significant effect not only at the biochemical level but at the organ and plant level additionally warrant future research on magnetic effects on live organisms.

In summary, the results of this study show inhibition of root growth by magnetic fields and a greater inhibition the stronger the magnetic field. We have also shown that the effect is quantitatively more important for seeds having a smaller diamagnetic signal. We have interpreted this observation by arguing that this is due to the lower magnetic susceptibility associated with the biological substances and cellular organelles such as the amyloplasts than to the cytoplasm bath, which creates an effective reduced magnetic force. We have also observed that the inhibition effect does not significantly progress as the root grows. We have suggested that this effect simply results from the progressive reduction of the magnetic force when moving away from the magnets. This effect provides additional evidence of the magnetic origin of the growth inhibition.

We conclude by noticing that the present experiments have been performed under magnetic fields that are in 10–100 G range, i.e., about two orders of magnitude more intense than the earth magnetic field (about 0.5 G). Environmental magnetic fields in residences are typically associated with power transmission lines (0.01–10 mG), ground currents (up to 5 mG), house wiring (0.01–10 mG) and appliances (5 mG–25 G). These fields are typically of 50–60 Hz and thus are essentially different than those used in this study (DC field); therefore, extrapolations cannot be made without risk. However, the present data conclusively show that a noticeable effect of the DC field exists on the root growth and thus it should stimulate new and more extensive studies on the effects of both DC and AC fields.

### ABBREVIATIONS

<table>
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<tr>
<th>Symbol</th>
<th>Description</th>
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<tbody>
<tr>
<td>$M$</td>
<td>Magnetization</td>
</tr>
<tr>
<td>$H$</td>
<td>Magnetic field</td>
</tr>
<tr>
<td>$\chi$</td>
<td>Magnetic susceptibility</td>
</tr>
<tr>
<td>$B$</td>
<td>Magnetic induction</td>
</tr>
<tr>
<td>ICAP-AES</td>
<td>Inductivity Coupled Argon Plasma Emission Spectrometer</td>
</tr>
<tr>
<td>$\rho$</td>
<td>Density</td>
</tr>
<tr>
<td>DC</td>
<td>Direct Current</td>
</tr>
<tr>
<td>AC</td>
<td>Alternate Current</td>
</tr>
</tbody>
</table>

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REFERENCES


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