Surface adhesion measurements in aquatic biofilms using magnetic particle induction: MagPI

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Running head: Magnetic Particle Induction

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Abstract

Sediment stability is a product of interacting physical and biological factors and while stability can be measured, few techniques allow sensitive assessment of the sediment surface as conditions change. For example, stability gradually increases as a biofilm develops, as salinity rises, or may be influenced by toxic compounds. This paper introduces a new technique (Magnetic Particle Induction: MagPI) based on the magnetic attraction of specially-produced fluorescent ferrous particles. The test particles are added to a surface and subjected to an increasing magnetic field, using either a permanent magnetic or a variable electromagnet. There is a strong correlation between magnetic flux density and distance from the surface (r^2 = 0.99) for the permanent magnets and for the magnetic flux density and the current supplied to electromagnets (r^2 >0.95). The magnetic force at which the particles are recaptured from the surface is determined as a measure of the adhesive nature of the surface. MagPI therefore determines the "stickiness" of the surface, whether a biofilm, a sediment or other material. The magnetic flux density (mTesla) required to remove test particles from diatom biofilms (mean 15.5 mTesla) was significantly greater than from cyanobacterial biofilms (mean 10 mTesla). Controls of fine glass beads showed little adhesion (mean 2.2 mTesla). Surface adhesion is an important bed property reflecting the sediment system's potential to capture and retain new particles and accumulate material. The methodology is dynamic, provides high precision, and is easily controlled. MagPI offers a straight forward and economic way to determine the surface adhesion of a variety of surfaces rapidly and with precision. The technique may have applications in physical, environmental and biomedical research.

Introduction

Biofilms are close to omnipresent in benthic aquatic systems and important in many scientific disciplines including medical research (Guo et al. 2008; Jain et al. 2007; Morton et al. 1998), waste-water treatment (Liu and Fang 2003; Raszka et al. 2006), toxicant removal (Sheng et al. 2008) and biotechnology (Flemming and Wingender 2001; Sutherland et al. 1998). Considerable interest has focused on the importance of biofilms for increasing sediment stability through the mechanical effects of the microbially-produced matrix of extracellular polymeric substances (EPS, reviewed in Stal 2003; Underwood and Paterson 2003). Sediment stability is a governing factor in sediment management because sediment transport and the release of associated contaminants have important consequences for the ecological and commercial health of aquatic habitats from the watershed to the sea (Förstner et al. 2004; Paterson et al. 2000). To assess the potential sediment erosion risk under hydrodynamic forcing, several devices have been developed to determine “the critical erosion threshold” (τ_{crit}) for sediment transport and the erosion rates (ϵ) of natural sediments. The critical erosion threshold is often an operational threshold defined by the characteristics of the chosen device rather than the actual theoretical point of incipient sediment motion (Tolhurst et al. 2000) This is partly because erosion devices are based on many different approaches including water flow (e.g. Sedflume, McNeil et al. 1996); SETEG, Kern et al. 1999), water jets (CSM, Paterson 1989), oscillation of a horizontal grid (Tsai and Lick 1986), a propeller (EROMES, Schuenemann and Kuehl 1991), or combined suction and flow (Gust-Microcosm, Gust and Mueller 1997).

While these methods all provide important information on the erosional behaviour of the sediment, they all require that bed failure occurs and therefore cannot measure any changes in surface properties below the point of incipient erosion. This restricts their use and because depositional beds must usually resist erosion to exist, this leaves a large gap in our knowledge and consequently more sensitive methods are needed. MagPI measures a different property of the sediment surface and thus does not replace erosion devices, but provides a framework for a sensitive analysis of surface adhesive capacity, a useful addition to the properties of the bed that can be determined.

The use of magnetism in various forms of bacterial biofilm research is widespread. Magnetic resonance imaging (MRI) has been used to visualise structure and detachment of biofilms (Manz et al. 2005; McLean et al. 2008), while surface bio-magnetism has been used to change cell adhesion and protein secretion (Chua and Yeo 2005). Immobilisation of magnetic particles by aggregates of pathogenic bacteria has been employed to assess biofilm formation in microtitre plates (Chavant et al. 2007). The new MagPI approach is based on the finding that the force needed to retrieve magnetic particles from a biofilm is a sensitive indicator of retentive ability or adhesive capacity of the substratum and a proxy for sediment “stability”. MagPI may also prove to be a possible index for other well-known features of a biofilm such as the potential to capture particulate-associated pollutants, binding of nutrients or the incorporation of deposited sediment particles, although this will require more research.

For the methodology demonstrated presented here, two types of magnets were used: permanent magnets and electromagnets. In both cases, a defined volume of magnetic particles (of known size range and density) were spread in a single layer onto a defined area of the submerged sediment surface and the force of the overlying magnet was increased until the particles were recaptured. The magnetic force was gradually enhanced by either reducing the distance between the magnet and the test particles (permanent magnet), or by increasing the electrical current to a variable magnet statically positioned 5-10 mm above the sediment (electromagnet). The sensitivity of this method has been illustrated by data presented for developing microalgal (diatoms and cyanobacteria) biofilms. The magnetic devices were found to be economically viable, easy to build and the Gauss Meter enables comparison of results gained in different devices/experiments/laboratories. The relative merits and the use of the two types of magnets (field, laboratory) are discussed.

Materials and Procedures

Magnetic particle induction

Permanent neodymium magnets— After extensive testing of a variety of permanent magnets, Neodymium alloy ($NdFe_3B$) disc-magnets were chosen for their superior magnetic strength, Nd being the most magnetic element found on earth (Coey 1995; Lebech et al. 1975). The Nd disc-magnets (20×5mm: e-magnets, UK) were applied either individually or as a stack of up to five, depending on strength requirements. Adding any more than 5 disc magnets did not increase the active magnetic field interacting with the test surface. The force from the permanent magnets acting upon the surface was regulated using decreasing distance to the bed controlled by an adjustable vernier-scaled manipulator (Fig. 1).

Electromagnets— The electromagnets were controlled by a precision power supply to allow fine and precise variation of voltage and current (Rapid 5000 variable power supply) (Fig. 1). A wide range of commercially available electromagnets was tested, but none showed the required functionality. The most common problems of commercial magnets were either in their size, obscuring the test surface or insufficient strength to retract the test particles from different surfaces. Thus, purpose-made electromagnets were constructed by using metal cores of ferrous alloy coiled with insulated copper thread. To increase the overall range of the magnetic field, two magnets were constructed covering a complementary range of magnetic forces. The magnets had metal cores of 10 mm and 5 mm diameters and were each coiled with 500 turns of copper thread with a diameter of 0.4 mm and 0.2 mm, respectively. The coil covered 60mm of the core on both magnets. The full coil resistance of the larger electromagnet was 35 Ω, and it was limited to an input range of 0-12 V (0-0.34 A). The smaller magnet had a coil resistance of 24 Ω and was limited to an input range of 0-20 V (0-0.83 A). Exceeding these limits burnt the coils, since above this level of supply increased current was dissipated as heat due to electrical resistance.

Ferrous test particles— The test particles consisted of an amalgam of ferrous material to provide a magnetic response, mixed with fluorescent pigment to increase their visibility (Fig. 2). An inert transparent binding agent combines the material into a solid which is then ground to produce a particle spectrum (Partrac, UK). The test particles were then sieved into different size classes. The size range selected for the trials was 180-250 μm, similar to fine/medium beach sand. The particles have to be applied to the test surface in consistent manner to allow repeatable measurements. To achieve a relatively even single layer of particles on the test surface took some practise but was achieved with experience. The test particles were suspended in water and the mixture drawn into a plastic pipette. The suspended particles were allowed to settle towards the tip of the pipette before being ejected as a single drop in the media above the test surface. A cut-off 2ml syringe, submerged into the water and held a short distance above the test surface, served as a guide to confine the particles to the selected test area.

Calibrations— To calibrate the device, the magnets were placed over a Hall sensor connected to a Gauss Meter (Unilab, Blackburn, England). The permanent magnets were lowered towards the probe in incremental steps (1 mm). The magnetic flux density (MFD) in mTesla was recorded for each step. For the electromagnets, the voltage and current was increased in small increments (0.2 V / ≈0.05 *A*) and the MFD for each increase was recorded. The Hall sensor calibrations were performed in air as well as submerged in water using a waterproof sensor. Calibrations were performed both before and after each experiment. During the experiment, the resistance of electromagnets was regularly checked. A decrease in coil resistance would be evidence of a fault which results in a loss of magnetic field strength.

Measuring procedure— The magnetic measurements were initiated immediately after the application of the particles. The magnet was lowered into position a set distance above the test surface. The distance to the test surface is critical and to ensure correct placement, a small guide rod (glass or plastic, not metal) was attached to the end of the magnet to insure the distance to the surface was set consistently (usually 10 mm). The magnet was lowered until the tip of the guide just contacted the test surface. This could be checked by use of a magnifying glass (in the field) or binocular microscope (in the laboratory). The magnetic field was increased in increments and four thresholds/levels of particle response were noted: (A) the particles show initial orientation (alignment) along the magnetic field; (B) the first particle is attracted to the magnet; (C) a small number of particles (around 5) are attracted to the magnet; (D) total removal of particles under the magnet. The third level is subjective and less reliable for a defined measurement. If the test surface is intended for repeated measurements, any stray particles deposited outside the test area should be cleaned from the surface with a permanent magnet to prevent compromising subsequent measurements.

Magnetic force equations— The attractive magnetic force (F) of a magnet is dependent on the magnetic flux density (MFD) and can be calculated according to:

$$F = \frac{B^2 A}{2\mu_0} \qquad \text{Equation 1}$$

where *B* is the MFD, *A* is the area of the magnet poles (in this case, the area of permanent magnet or electromagnet that faces the surface) and μ_0 is the permeability of the free space (Breithaupt 1999a) which is a constant during measurements in the same medium.

The magnetic force (F) that an electromagnet can produce at the pole surface is calculated according to:

$$F = \frac{\mu^2 N^2 I^2 A}{2\mu_0 L^2} \qquad \text{Equation 2}$$

where μ is the permeability of the core material, N is the number of thread turns in the coil, I is the current, A and μ_0 as above, and L is the full length of the thread used in the coil (Breithaupt 1999b). Consequently, the magnetic force (F) can be controlled by varying the current (I) while all other factors are held constant.

Precision and Statistics— The precision of the method was tested through repeated calibrations (n=25). Based on 95 % confidence intervals, an average precision of 0.1% for the electromagnet measurements was determined (±0.22 % in the low current range, ±0.035%, in the mid current range and ±0.045 % in the high current range). The use of a different electromagnet and/or other power source requires a separate precision test to be conducted, but as long as a suitably sensitive power supply is used, a similar range could be expected. Data was assessed for normality and homogeneity of variance and then a one-way ANOVA was applied (significance level of $\alpha = 0.05$) and post-hoc test (Tukey) to determine differences in surface adhesion between varying surfaces and biofilm compositions.

Calibration results— There were strong linear relationships ($r^2 = 0.996$-0.997) between current (I) and the magnetic flux density (mTesla) for the electromagnets. The relationship between distance and magnetic field strength of the permanent magnets was exponential (Fig. 3). In contrast to the electromagnets, the permanent magnets have to be moved towards the surface during the measurement to increase F. Consequently, the area of the magnetic field that interacts with the surface increases with decreasing distance and this corresponds to a non-linear increase of the field strength (Fig. 3a). The line of best fit for the calibration of the permanent magnet strength versus distance required a sixth order polynomial as opposed to the linear function used for the electromagnet calibration (Fig. 3a).

Abiotic particulate surfaces—Substrata of different types and particles sizes were tested during the pilot studies: two size fractions of clean glass beads (<63µm and >150µm Ballotini™ beads), as well as sand and mud which had been furnaced to remove organic material. These substrata were submerged in both seawater and freshwater to take into account any ionic interactions.

Biofilm surface testing— The influence of biotic surfaces was examined using cultured biofilms of benthic cyanobacteria (dominated by *Oscillatoria* spp.) and pennate diatoms (dominated by *Nitzschia* spp.). Both cultures were grown on clean glass beads (<63µm Ballotini™) in a temperature-controlled room (15°C) under a 13 h/11 h light/dark cycles (~250 µmol m^{-2} s^{-1}). Similar glass beads covered with pre-filtered (1 µm), autoclaved seawater without microalgal inoculums served as a control. For the treatments and the controls, plastic weighing trays (55 × 55 × 23 mm) were filled with a 5mm layer of the <63 µm glass beads and filled with autoclaved seawater. The experimental period covered 19 days to follow changes in the surface properties of developing biofilm cultures. The small electromagnet described above was employed for these tests.

Threshold conditions— In terms of the thresholds of test particles response to magnetic force, the total clearance (D) was the preferred measure. Firstly, this threshold is the least subjective and the data gained by different persons are almost identical and secondly, this threshold showed significant differences between treatments, which were not always obvious using the other three thresholds (Fig. 4). Under laboratory condition, where more sophisticated observation using microscopy of the particles is possible, the first and second threshold can be used as an alternative and/or complementary value. In general terms, we recommend recording all thresholds if possible as each may indicate a slightly different property of the surface.

Assessment

Abiotic particulate surfaces— The force required to recapture the test particles (size 180-250 μm) from various substrata are given (Fig. 5). Measurements indicated differences between seawater and freshwater conditions. Under seawater, it was more difficult to capture test particles from the bed composed of larger glass bead than from the smaller glass beads, followed by mud and then the cleaned sand (Fig. 5a). Under freshwater conditions, the magnetic force needed to retrieve the test particles was similar for all surfaces except the larger glass beads which showed a significantly higher “retentive capacity” (Fig. 5b).

For the sand, similar forces were needed to retrieve particles in seawater and freshwater, but relatively greater force had to be applied in seawater to recapture particles from the other substrata (compare Fig. 5a and b). This is probably due to the ionic nature of seawater increasing the potential for electrostatic and other physico-chemical attractions between particles (e.g. mud with silt and clay content known for their surface charge variation). This could imply that the ionic milieu facilitates the cohesion of the surface as measured by MagPI. However, increased binding capacity was also noted in freshwater from the larger glass bead substratum. This may be because the magnetic particles become physically trapped in the pore spaces between the larger glass beads. However, both the smaller and larger glass beads showed enhanced surface cohesion in seawater as opposed to freshwater which suggests both mechanisms may be responsible for the binding capacity of the larger glass beads.

Biotic experiment example— The biotic test experiments revealed that the biofilms developed by benthic diatoms under these conditions had a more adhesive surface compared to the cyanobacterial biofilms (Fig. 6). One plausible explanation for this was that the experimental irradiance was relatively high and cyanobacteria, in this case dominated by

Oscillatoria spp., tend to prefer lower light levels, thus forcing them deeper into the sediment matrix and reducing surface EPS production. Diatoms, in contrast, are better adapted to higher irradiances. The important aspect was that the MagPI method was able to detect even quite small differences in surface adhesion with high precision (Fig. 6).

Discussion

Application and replication of the method— The equipment required for the method described here is simple and affordable (Figs. 1 and 2). However, production of suitable electromagnets does demand some technical understanding to achieve the acquired magnetic strength.

In the laboratory, electromagnets were preferred over permanent magnets due to the accuracy of their calibration and ease of deployment. Depending on the design and power source, electromagnets offer the possibility to increase the magnetic force in small steps, thus offering a high resolution within the applied magnetic strength range. A fixed distance marker (non-metallic) fitted at the tip of the electromagnet helps to ensure positional accuracy between measurements. Permanent magnets are recommended for measurements in the field (e.g. tidal flats) because of the logistical ease for field use. The permanent magnets still produce an accurate and stable force at each set distance, although the precise manipulation of the distance between the magnet and the test surface is critical. To ensure correct initial placement, a small guide rod (glass or plastic, not metal) was used to set the magnet the desired initial distance from the surface (cf 2 cm). The vernier scale ($\pm$ 100 µm) was then used to move the magnet in small incremental steps and the results recorded. The test surface must be reasonably flat and the magnet face set parallel to the surface.

Ferrous test particles — The choice of the size of the test particles is an important decision. It is sensible to select a size range of particles that does not deviate too much from the test sediment, preferably being slightly larger to prevent trapping in surface pore space. Although this type of trapping may not be an issue on surfaces where biofilm has developed, the test particle size is also important for the ease of observation on the surface. It is also sensible to use a narrow size range of test particles, to enhance the uniformity of the particle interactions with the surfaces.

Another variable is the "incubation" time or period that particles are left on the test surface before performing the measurement. Since this depends on the characteristics of the investigated surface as well as on the objectives of a particular study, it should be decided by the operator on the basis of the question to be addressed in each experiment. The simplest way to ensure a repeatable measure of the test surface is to retract particles directly after their addition and the most appropriate value of the surfaces "stickiness" can be gained directly after adding the particles. When particles are left for a longer time, they will be partly or fully incorporated in the biofilm and the measured variable becomes a combination of the adhesion of the surface and the capacity to entrap particles by biofilm development (Fig. 2 E). However, we can envisage experiments (and have begun to conduct them) where particles are added and time after addition is an important variable of interest.

Comments and recommendations

Advantages and limitations— A great advantage of MagPI is the ability to measure biofilm adhesion, a variable that has rarely been considered, but is at the same time of great significance for binding pollutants, trapping nutrients, enhancing sediment stability and capturing new deposited particles. For instance, the "ecosystem service" (Paterson et al. 2008) of particle capture and retention is of great importance to sediment systems in balancing the replacement of material lost by tidal erosion (Verney et al. 2006) or wave action (Andersen et al. 2007), enhancing the nutrient status (Freeman and Lock 1995) and offering binding sites for pollutants (Ghosh et al. 2003). This biofilm adhesion can be measured with high sensitivity and small changes in developing biofilms can be demonstrated which would be unnoticed using established erosion devices. MagPI comes at comparatively low cost, and with basic practical skills and technical understanding it is comparatively easy to build and use.

Although the permanent magnet is valuable for the use in the field, MagPI cannot easily be used if a wet biofilm is not submerged, such as during tidal emersion periods. The measurements have to be performed underwater by the help of a water-filled chamber, otherwise the magnetic particles interact with the surface tension of the water-film and these forces confound the measurement of adhesion.

Other possible applications— This method can be used for any sub-tidal or intertidal sediments, including complex biofilm-based systems such as stromatolites (Paterson et al. 2008) but the measurements of moist surfaces should be made underwater because of surface tension effects. In addition, dry exposed surfaces where adhesion is important might also be examined, to-date we have tested very few other substrata, but stonework, tree surfaces, leaves etc remain possible candidates for investigation.

The MagPI represents an economically viable, easily constructed, easy-to-use tool to determine surface adhesion, a proxy for the retentive capacity of the substratum. The knowledge of surface adhesion can provide useful insights for particulate pollutant capture, nutrient trapping, enhancing sediment stability and capturing particles in various depositional systems such as intertidal flats, shallow submerged sediment systems and stromatolites to name but a few. In contrast to established erosion devices, MagPI can determine small changes in surface properties below the point of incipient erosion with high sensitivity, high accuracy and a high repeatability. The calibration by the Gauss meter makes the comparison of the data between different experiments and various laboratories possible, which is an important prerequisite for future success in biofilm research. Two types of magnets have been examined here: the high power permanent magnet for increased mobility and application in the field and the electromagnet which is to be preferred in the laboratory due to a higher accuracy in calibration and measurement. The technique presented here is likely to have future applications in environmental, medical and biotechnological research.

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<u>Figures and figure legends</u>

Figure. 1. Schematic diagram of the two variants of MagPI. Electromagnetic version (left) with a variable current supply and permanent disc-magnets (right) using decreasing distance to surface. In each case, fluorescent ferrous particles are added to the sediment surface. The current input or the distance from the surface is recorded in each case, respectively, as the particles respond.

Figure. 2. A: Prototype of MagPI placed above the surface of a sample of intertidal microbial sediment (stromatolite) during the NSF RIBS programme (see acknowledgements). Test particles can be seen adhering to the magnet. B: Sample of stromatolite prepared for MagPI measurement. C: Detail of surface showing fluorescent particles among stalked diatoms on one region of stromatolite. D and E: Confocal microscopy of fluorescent beads on the stromatolite surface becoming incorporated into the biofilm. The green colouration represents organic material while the red fluorescence represents the test particles. Note the test particles are approximately 125-150 µm across in this example. (Bar markers: A = 1 cm, B = 5 cm, C = 5 mm, D = 150 µm, C = 150 µm). Confocal images supplied by Dr A. Decho.

Figure. 3. Examples of calibration curves for the permanent (A) and electromagnetic (B) devices, respectively. A: An exponential increase in force as the permanent magnet approaches the surface. B: The strong linear relationship between magnetic force and supplied current for the electromagnet as the current was gradually increased. Black line- the magnet was stationed 5 mm from the surface. Grey line- the magnet was stationed 10 mm from the surface.

Figure. 4. The thresholds used in the magnetic measurements (A) particle orientation to magnetic field, (B) first magnetic particle captured by the magnet, (C) larger groups of particles attracted and finally (D) total clearance of particles under the magnet. Three treatments are given as examples: Small glass beads submerged in seawater (SW) and freshwater (FW) and large glass beads in SW using test particles of size range 180-250 µm. n=6, SE-bars.

Figure. 5. Tests on abiotic particulate beds of different materials in (a) seawater and (b) freshwater to attract test particles (180-250 µm) by MagPI (n = 6, ±SE, * indicates significant difference between adjacent groups by ANOVA, $\alpha = 0.05$ and subsequent Tukey test)

Figure. 6. Biotic example with cultured biofilms grown with diatoms and cyanobacteria, respectively. The cyanobacterial biofilm had a lower surface adhesion than the diatom biofilm. The threshold reported is the strength of the magnetic field needed to provide total clearance of particles under the magnet (n = 6, ±SE, * indicates significant difference between groups by ANOVA, $\alpha = 0.05$ and subsequent Tukey test)

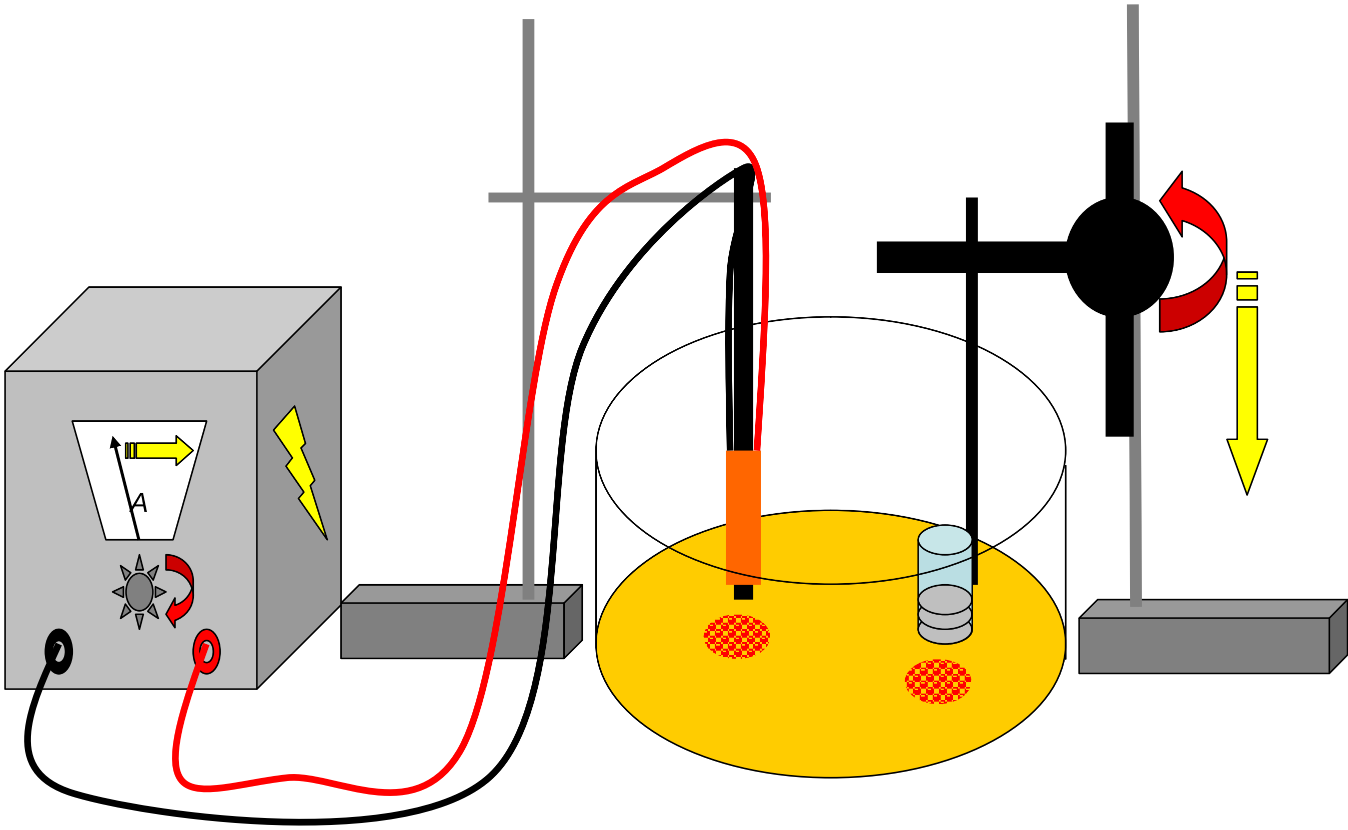
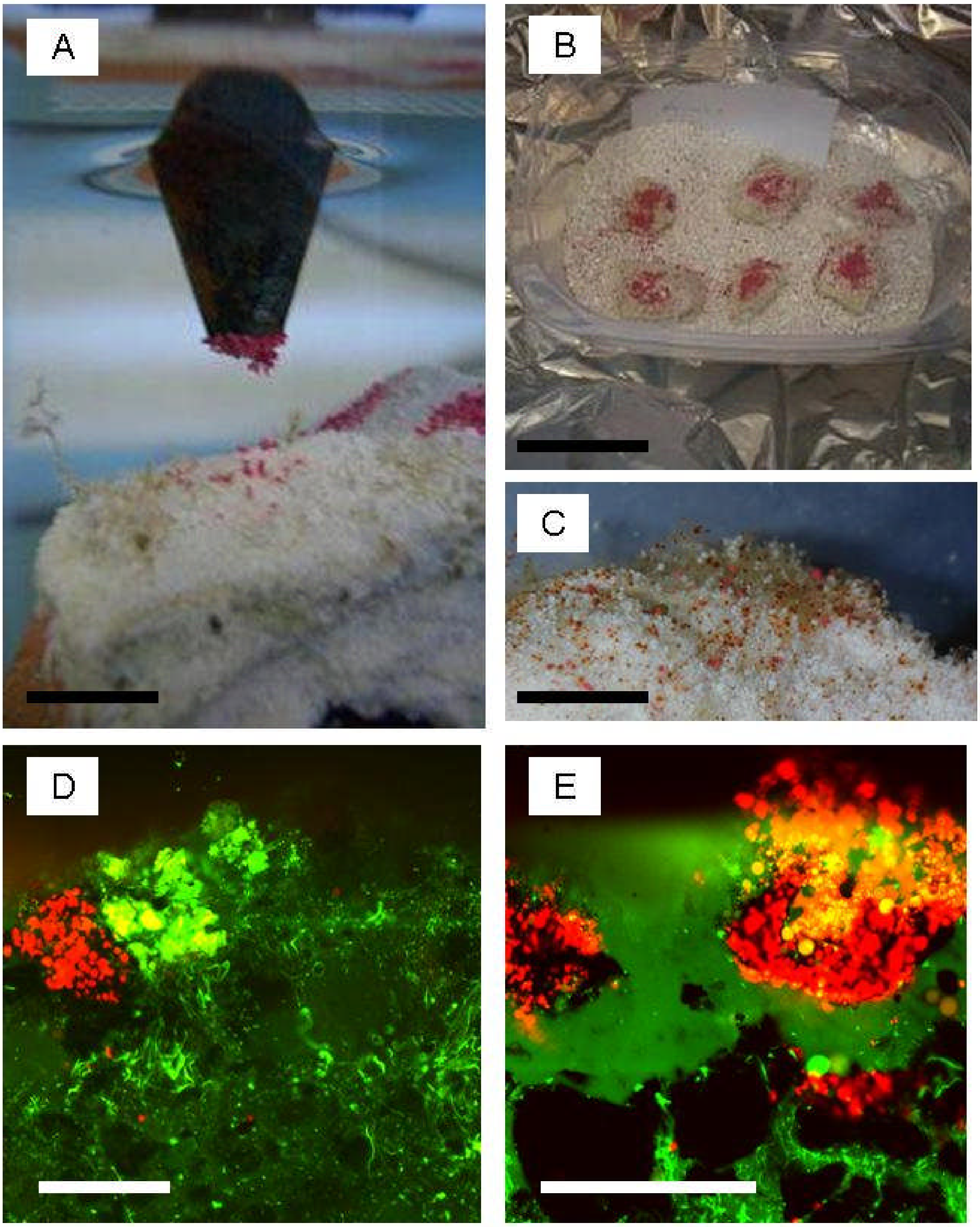


Fig. 1. Larson et al



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472 Fig. 2. Larson et al

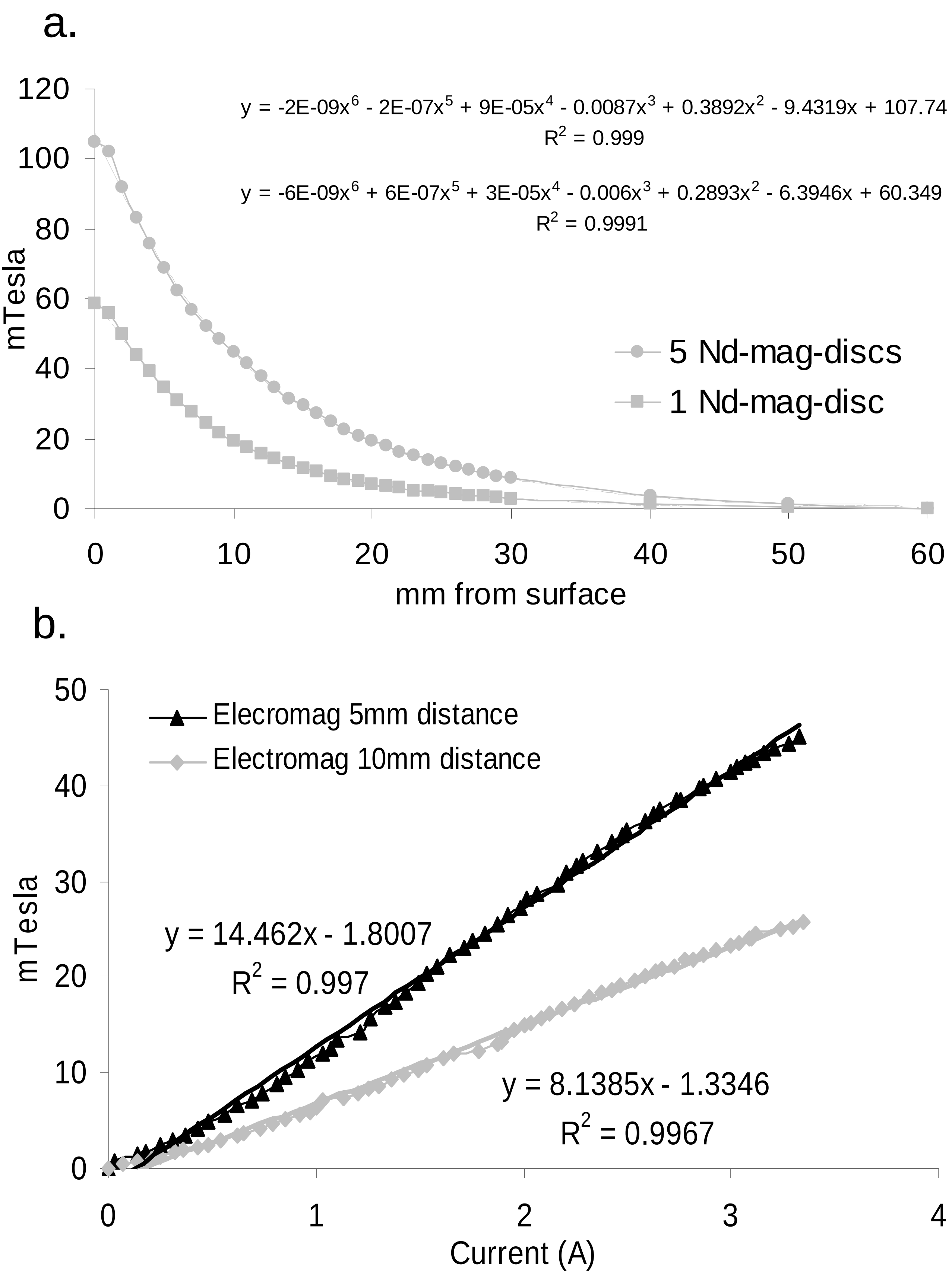

Fig. 3. Larson et al

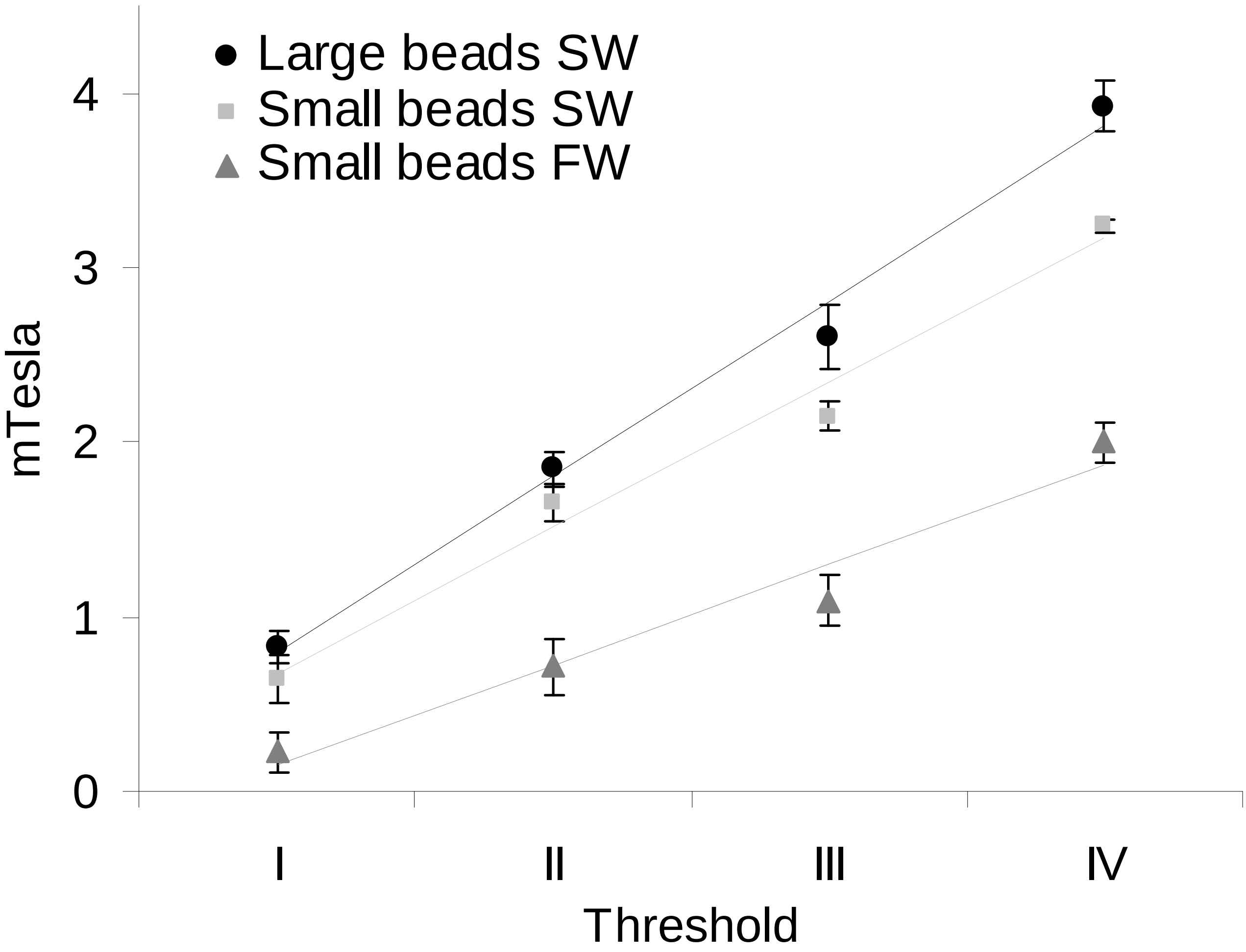

Fig. 4. Larson et al

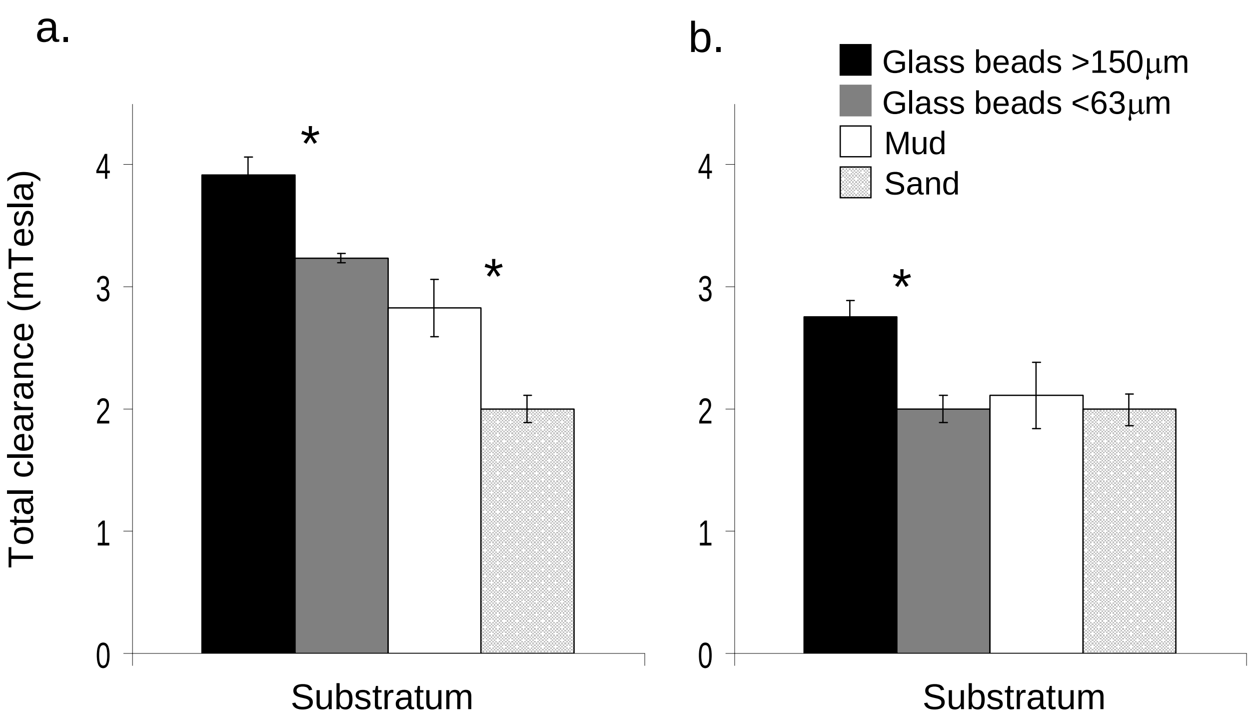

Fig. 5. Larson et al

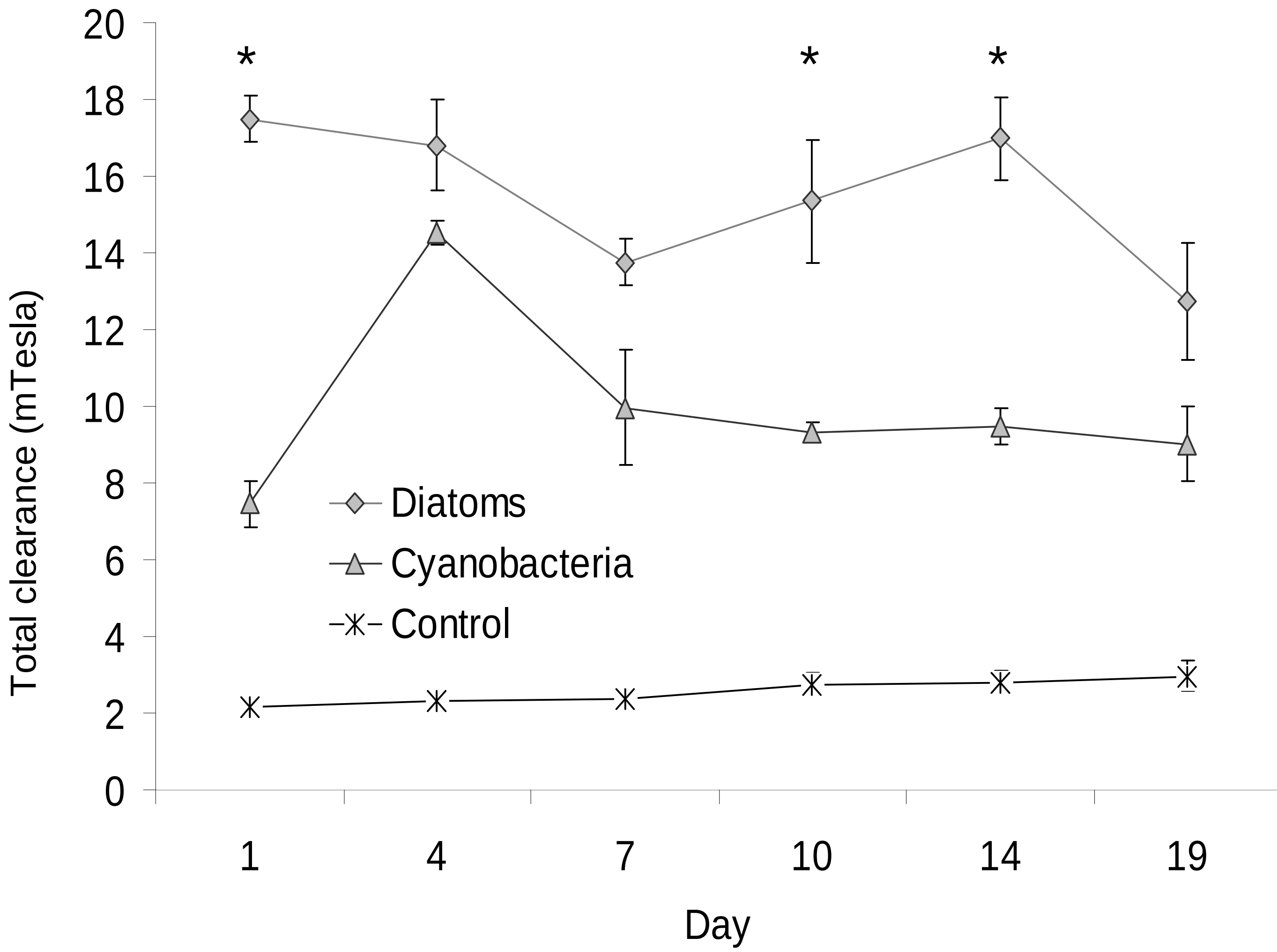

Fig. 6. Larson et al