EFFECT OF HEAVY METAL CATIONS ON THE FATE OF EXTRACELLULAR DNA ADSORBED AND BOUND ON CLAY MINERALS

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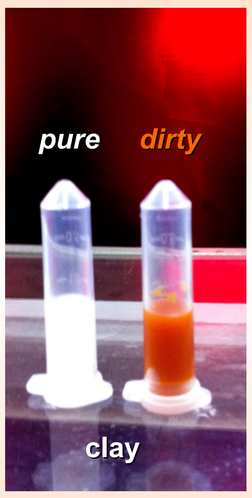
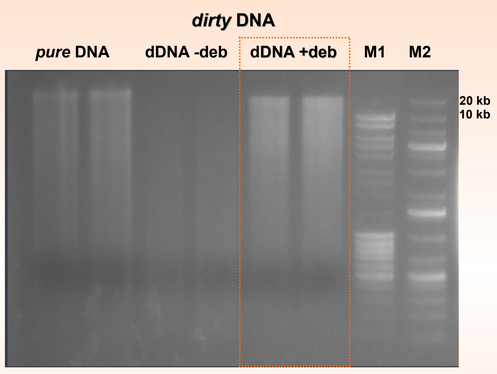
The presence of high-valent metal cations on clay mineral surfaces is hypothesised to induce conformational changes in the secondary and tertiary structure of the DNA molecule adsorbed and bound onto clays, defined as M-conformation, and its condensation. The hypothesis that these reversible phenomena could enhance the resistance of DNA to enzymatic degradation strongly encourages the studies on the effects of heavy metal contamination in clay rich soils on the fate of extracellular soil DNA (eDNA). This lack of knowledge is relevant concerning the ecological role of soil eDNA in terms of persistence and availability for bacterial horizontal gene transfer by natural transformation, and as substrate for biofilm formation (Pietramellara et al., 2009).

We assessed the effect of ionic Fe polymers on the adsorption (loosely *versus* tightly bound) of eDNA on clay minerals (*dirty* clay), that represent the mineral fraction of soil colloids. DNA-clay complexes (*dirty* eDNA/pure clay versus *dirty* eDNA/*dirty* clay) were analysed in terms of strength of DNA-clay interaction (adsorption isotherms). Challenging to conduct DNA-clay interaction studies in conditions as natural as possible, *dirty* eDNA was extracted by simulating natural cell lysis without any purification, in order to avoid possible bias coming from DNA purification processes.



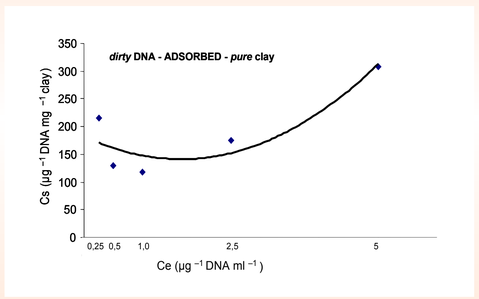


Molecular weight of differently pure DNA fractions assessed by agarose gel electrophoresis. *Dirty* DNA (dDNA) coated with cellular debris (+deb) was used for interaction studies with *pure* clay (Ca^{2+}- M) and *dirty* clay (Ca^{2+}-M coated with ionic Fe). M1, M2 are standardized DNA markers.

- ***pure* clay: Ca^{2+}-Montmorillonite (M; Wyoming <2µm; Fusi et al., 1989).**
- ***dirty* clay: Ca^{2+}-M coated with $Fe(NO_3)_3$ (Oades, 1984).**
- ***dirty* DNA was extracted from *Bacillus subtilis* BD1512 by modifying the protocol of Svarachorn et al. (1989) and classified as *dirty* DNA with cellular debris (dDNA+deb; Pietramellara et al., 2007).**
- ***Adsorption isotherms* were performed with 0.25 mg of a) *pure* and b) *dirty* clay, by adding 0.25, 0.5, 1.0, 2.5, 5.0 µg of *dirty* DNA (Pietramellara et al., 2007).**

The fractions of DNA 1) *not adsorbed*, 2) *loosely bound* and 3) *tightly bound* on clay minerals were determined based on fluorimetry (Qubit). The adsorption isotherm data were fitted to the Freundlich and Langmuir equations.

Preliminary results



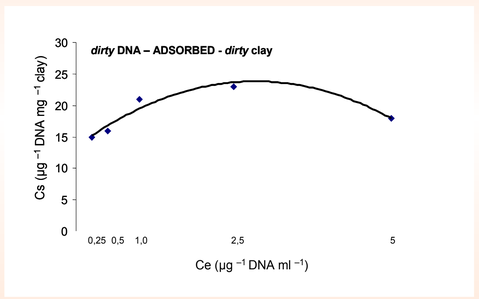
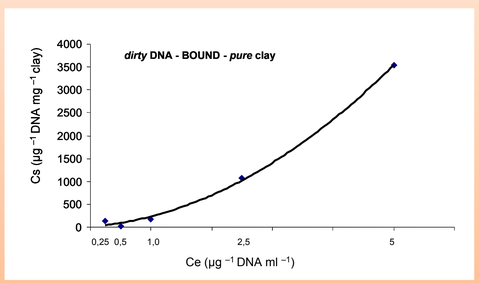


Fig. 1 Adsorption isotherms of *dirty* DNA and *pure* versus *dirty* clay showing different binding behaviors (*loosely bound*) as function of absence and presence of Fe polymers on clay mineral surfaces.



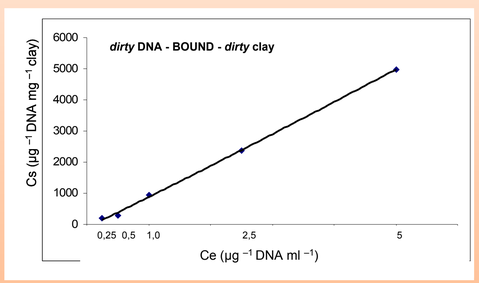
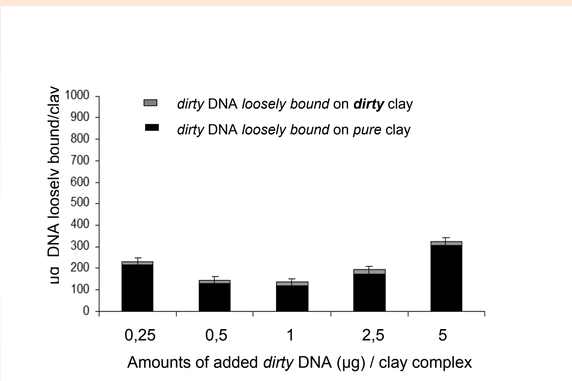


Fig. 2 Binding behavior (*tightly bound*) of *dirty* DNA on *pure* versus *dirty* clay as function of absence and presence of Fe polymers on clay mineral surfaces.



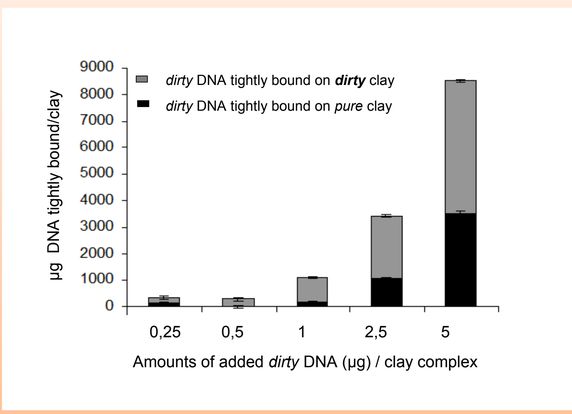


Fig. 3 Amounts of loosely and tightly bound dirty DNA on *pure* versus *dirty* clay.

DNA-clay interaction studies have been mostly performed under *artificial conditions* (*pure* DNA/*pure* clay). The performance under *more natural conditions* (*dirty* DNA/*pure* clay) suggested a *positive effect* of cellular debris coating DNA on DNA adsorption behaviors (Pietramellara et al., 2007).

The present study, simulating *natural soil conditions* (*dirty* DNA/*dirty* clay), provided evidences of different amounts of DNA loosely (Fig. 1) and tightly bound (Fig. 2) on *pure* and *dirty* clay minerals, respectively. Lower amounts of loosely and higher amounts of tightly bound DNA on *dirty* clay with respect to *pure* clay have been observed (Fig. 3), suggesting Fe polymer-induced differences in the strength of interaction of DNA with clays.

The proposed approach is capable to address a so far neglected aspect of heavy metal (HM) pollution, especially of clay-rich soils, namely the impact of HM cations coating soil colloid surfaces on the fate of eDNA in soil, providing relevant information on risk assessment of HM contaminated soils.

Fusi et al. (1989) *Soil Biol Biochem 21:911-920;* ***Oedas (1984)*** *Clays and clay minerals 32:49-57;* ***Pietramellara et al. (2007)*** *Biol Fertil Soils 43:731-739;* ***Pietramellara et al. (2009)*** *Biol Fertil Soils 45:219-235;* ***Svarachorn et al. (1989)*** *Appl Microbiol Biotechnol 30:299-304.*