Pediatric Acute Lung Injury (ALI) is associated with a high mortality and morbidity, and dysregulation of matrix metalloproteinases (MMPs) may play an important role in the pathogenesis and evolution of ALI. Here we examined MMP expression and activity in pediatric ALI compared with controls. MMP-8, -9, and to a lesser extent, MMP-2, -3, -11 and -12 were identified at higher levels in lung secretions of pediatric ALI patients compared with controls. Tissue Inhibitor of Matrix metalloproteinase-1 (TIMP-1), a natural inhibitor of MMPs was detected in most ALI samples, but MMP-9:TIMP-1 ratios were high relative to controls. In subjects who remained intubated for .10 days, MMP-9 activity decreased, with > 80% found in the latent form. In contrast, almost all MMP-8 detected at later disease course was constitutively active. Discriminating MMP-9:TIMP-1 ratios were found in those who had a prolonged ALI course. These results identify a specific repertoire of MMP isoforms in the lung secretions of pediatric ALI patients, and demonstrate inverse changes in MMPs -8 and -9 with protracted disease.